FORM PTO-1390 (REV 11-98)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 39-187
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S APPLICATION NO (If known, see 37 C.F R. 1.5) 09/367261 Unknown
INTERNATIONAL APPLICATION NO. **PCT/GB98/00461**	INTERNATIONAL FILING DATE **13 February 1998**	PRIORITY DATE CLAIMED **13 February 1997 10 June 1997**

TITLE OF INVENTION

DRUG TARGETING

APPLICANT(S) FOR DO/EO/US

BLAKE et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☒ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   - d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information. PTO-1449 and International Search Report

510 Rec'd PCT/PTO 13 AUG 1999

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/367261 Unknown	PCT/GB98/00461	39-187

17. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY		
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):			
-- Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$970.00			
-- International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00			
-- International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2) paid to USPTO.....$760.00			
-- International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00			
-- International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00			
ENTER APPROPRIATE BASIC FEE AMOUNT =	$	840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	130.00	

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE			
Total Claims	**25**	-20 =	5	X	$18.00	$	90.00
Independent Claims	**2**	-3 =	0	X	$78.00		0.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)				+$260.00		$	0.00
				TOTAL OF ABOVE CALCULATIONS =		$	**1060.00**
Reduction by ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).							0.00
				SUBTOTAL =		$	**1060.00**
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				+			0.00
				TOTAL NATIONAL FEE =		$	**1060.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property				+		$	0.00
Fee for Petition to Revive Unintentionally Abandoned Application ($1,210 – Small Entity Fee = $605)						$	0.00
				TOTAL FEES ENCLOSED =		$	**1060.00**
					Amount to be: refunded	$	
					charged	$	

a. ☒ A check in the amount of $1060.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Mary J. Wilson
SIGNATURE

Mary J. Wilson
NAME

32,955 **August 13, 1999**
REGISTRATION NUMBER Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

BLAKE et al Atty. Ref.: **39-187**

Serial No. **Unknown** Group:

Filed: **August 13, 1999** Examiner:

For: **DRUG TARGETING**

* * * * * * * * * * *

August 13, 1999

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Examination of this application should be initiated based on the claims as amended under PCT Article 34. Prior to calculation of the filing fee and in order to place the above identified application in better condition for examination, please amend the claims as follows:

IN THE CLAIMS

Claim 4, line 1, delete "or claim 3".

Claim 5, line 1, delete "any one of claims 1 to 4" and insert -- claim 1 --.

Claim 13, lines 1 and 2, delete "any one of claims 1 to 5" and insert -- claim 1 --.

Claim 15, lines 1 and 2, delete "any one of claims 1 to 5" and insert -- claim 1 --.

Claim 16, lines 1 and 2, delete "any preceding claim" and insert -- claim 1 --.

Claim 19, lines 1 and 2, delete "any one of claims 1 to 18" and insert -- claim 1 --.

Claim 20, line 2, delete "any one of claims to to 18" and insert -- claim 1 --.

Claim 21, line 1, delete "any one of claims 1 to 18" and insert -- claim 1 --.

Claim 22, line 1, delete "any one of claims 1 to 18" and insert -- claim 1 --.

Claim 23, line 1, delete "any one of claims 1 to 18" and insert -- claim 1 --.

Claim 25, line 3, delete "any one of claims 1 to 19" and insert -- claim 1 --.

REMARKS

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: [signature]
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Applicant or Patentee: David Blake et al — Case Docket No. ____________
Serial or Patent No: ____________
Filed or Issued: ____________
For: DRUG TARGETING

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9 (f) and 1.27 (b)) - SMALL BUSINESS CONCERN

I hereby declare that I am

( ) the owner of the small business concern identified below.
(X) an official of the small business concern empowered to act on behalf of the concern identified below.

NAME OF CONCERN: Theramark Limited
ADDRESS OF CONCERN: 90 Fetter Lane, London, EC4A 1JP, United Kingdom

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled DRUG TARGETING ____________ by inventor(s) ____________ described in

( ) the specification filed herewith
( ) application serial no. ____________, filed ____________
( ) patent no. ____________, issued ____________

If the right held by the above identified small business concern is not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

FULL NAME ____________
ADDRESS ____________
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

FULL NAME ____________
ADDRESS ____________
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING DR. EDWARD JOHN RUSSELL-DUFF
TITLE OF PERSON ON BEHALF OF THERAMARK LIMITED DIRECTOR.
ADDRESS OF PERSON SIGNING Watch Lane Farm, Moston, Sandbach Cheshire CW11-3QS United Kingdom
SIGNATURE Edward John Russell-Duff DATE 23 August 1999

DRUG TARGETING

The present invention relates to bioreductive drug conjugates for use in targeting of therapeutic agents to localised regions of hypoxic and/or ischemic tissue within the body.

Reduced oxygen tension (hypoxia) has been demonstrated in a variety of tumor types. In fact, it has long been suspected that oxygen deficiency in tumors may be a limiting factor in the control of tumors by radiotherapy. Relatively recently, the presence of hypoxia in tumors has been exploited in their treatment.

Bioreductive drugs require metabolic reduction to generate cytotoxic metabolites. This process is facilitated by the presence of appropriate reductases and the lower oxygen conditions present in some cancerous (hypoxic) as compared to normal (normoxic) tissue. As a result, a number of bioreductive drugs capable of producing cytotoxic metabolites under hypoxic conditions have been proposed for use in combination with radiotherapy treatment of tumors.

A number of bioreductive compounds are known to act as potent alkylating agents after undergoing reduction *in vivo*. Examples of known bioreductive alkylating agents include compounds such as activated enamines, vinylogous quinone methides, simple quinone methides and α-methylene lactones or lactams. Bioactivation of such compounds produces species which are electron deficient and which are capable of covalent binding to a nucleophilic centre on a biomolecule, such as DNA.

Most bioreductive drugs that have been developed for use in the treatment of tumors exhibit an optimum "trapping" potential when hypoxia is profound (pO_2 < 12 mm Hg) and this is believed to form the basis for their selectivity for cancerous as opposed to normal tissues.

Bioreductive drugs have also been proposed for use

in several methods for the detection of hypoxic cells in tumors. In this way, radiotherapy treatment may be optimised for individual patients on the basis of the oxygen status of their tumors.

US-A-5086068 describes the use of nitroaromatic compounds in the detection of hypoxic cells in normal and tumor tissue. An immunogenic conjugate comprising a nitroaromatic compound and an immune response inducing carrier is used *in vitro* to raise antibodies specific to the nitroaromatic compound. These antibodies are in turn used to detect the presence of hypoxic tissue following *in vivo* administration of the nitroaromatic compound.

A number of methods have also been described for detecting the presence of hypoxic cells in tumors using a labelled 2-nitroimidazole in which labelled fragments of the nitroimidazole compound bind to cellular macromolecules. More recently, the use of an immunologically detectable hapten such as theophylline covalently bound to a 2-nitroimidazole has been suggested as a method of indentifying hypoxic cells (see Brit. J. Cancer 63: 119-125, 1991 & 72: 1462-1468, 1995, and Anti-Cancer Drug Design 10: 227-241, 1995). Bioreduction of the nitroimidazole leads to binding of bioreductive metabolites, and hence the theophylline side-chain, to intracellular molecules. Immunochemical techniques are then used to stain and thus locate those cells containing the bound theophylline.

Other agents comprising a bioreductive moiety, e.g. 2-nitroimidazole, for the diagnosis or treatment of hypoxic cells are described in US-A-5387692.

A number of bioreductive agents have been described for use in the delivery of cytotoxic drugs to hypoxic tumor tissue in which bioreductive activation at the tumor site results in selective delivery of the drug. However, following drug delivery the bioreductive compound remaining in the tissues is itself a potential

alkylating agent and thus cytotoxic, thereby rendering such a system entirely unsuitable for use as a non-cytotoxic drug delivery vehicle in diseases other than cancer. Hypoxia-selective bioreductive drug delivery agents proposed for use in anti-tumor therapy are described, for example, in Dissabs. 87: 31004, 1987 and in J. Med. Chem. 34: 2933-2935, 1991.

Delivery systems which utilise bioreduction to deliver a non-cytotoxic drug species have also been proposed. For example, a delivery system based on quinone propionic acid has been described (see Pharmaceutical Research 8(3): 323-330, 1991) in which the benzoquinone acts as the trigger and the propionic acid moiety allows for linkage either to an amine moiety (e.g. an enzyme inhibitor) or to an alcohol (e.g. a steroid). Two electron activation of the benzoquinone trigger facilitates intramolecular cyclisation generating a stable lactone, a process which results in elimination of the drug species. However, the lactone produced is itself a potential alkylating agent. This system is thus unsuitable for use as a non-cytotoxic drug delivery system. Furthermore, in aqueous solution in the absence of a reducing agent the lactone produced following drug delivery is very unstable and undergoes degradation. The instability of this prodrug system in aqueous solution thus precludes its use for drug delivery *in vivo*.

We now propose an improved method for the specific targeting of a drug to areas of hypoxic and/or ischemic tissue, e.g. cells, tissues and/or organs, within the body in which the desired drug species is linked to a non-cytotoxic bioreductive compound or carrier. In this method, any direct interaction of the carrier with DNA or other biomolecules is minimised, thus avoiding potential mutagenic side effects.

In particular, we now propose a method capable of targeting drugs to sites of inflammation within the body

associated with hypoxia and/or ischemia, e.g. to the synovium in the treatment of rheumatoid arthritis. This method not only has the effect of reducing the risk of systemic side effects of the drug, but also enhances the therapeutic effect of the drug.

Thus, viewed from one aspect the invention provides a bioreductive conjugate comprising a non-cytotoxic bioreductive moiety with linked thereto at least one therapeutic agent.

The bioreductive conjugates in accordance with the invention are substantially stable in an oxygenated environment. However, in a hypoxic or ischemic environment, reductive activation results in release of the therapeutic agent from the bioreductive moiety and thus its targeted delivery to the site of hypoxia or ischemia which may be an organ, tissue, cell or group of cells. In general, on bioreduction the bioreductive moiety will undergo an intramolecular rearrangement or intramolecular cyclisation reaction which in turn provides for release of the therapeutic agent at the target site.

As used herein, the term "bioreductive moiety" is intended to define any molecule which is reduced in the presence of reducing enzymes or reductases. For example, a bioreductive moiety may be any substantially non-reactive molecule which in the presence of reductases is converted into a more reactive form. Preferred bioreductive moieties for use in the invention are those which on reductive activation become electron-rich and which are thereby capable of intramolecular bond rearrangement to deliver a therapeutic agent.

As used herein, "non-cytotoxic bioreductive moiety" is used to define any bioreductive moiety having substantially no cytotoxic activity *in vivo*. Thus, it is intended that the bioreductive moiety for use in accordance with the invention is not only in itself non-cytotoxic, but that this produces substantially no

cytotoxic species following bioreductive activation. By "non-cytotoxic" it is meant that the bioreductive moiety does not interact directly with DNA. Preferably, the bioreductive moiety is substantially non-mutagenic. Thus, the bioreductive moiety is intended to function merely as a non-cytotoxic carrier or targeting agent for the drug species which, following delivery of the drug at the target site, is eliminated from the body in the absence of any undesirable side-effects.

The bioreductive conjugates in accordance with the invention have a targeting effect on tissues having reductase activity. This is believed to be a consequence of hypoxic metabolism and/or reduced oxygenation of such tissues.

In one embodiment the invention provides bioreductive conjugates of formula (I):

$$A(B)_n \qquad (I)$$

where A is a non-cytotoxic bioreductive moiety, each B is independently the residue of a therapeutic agent, and n is an integer, preferably from 1 to 3, particularly 1.

A and B are stably conjugated in an oxygenated environment and are such that A is non-cytotoxic and B when conjugated to A is non-cytotoxic. On reductive activation of A, A and B detach and A is itself either a stable, non-cytotoxic species or, more preferably, A reacts with itself to form a stable, non-cytotoxic species.

Preferred compounds for use in accordance with the invention are those which have the ability to penetrate poorly perfused tissues and which only release the active drug in a hypoxic and/or ischemic environment.

A large number of bioreductive agents of diverse structure are known. These include quinones, aromatic nitro compounds and N-oxides. As mentioned above, those intended for use in accordance with the invention should

be substantially non-cytotoxic following bioreductive activation. This may be achieved in a number of ways.

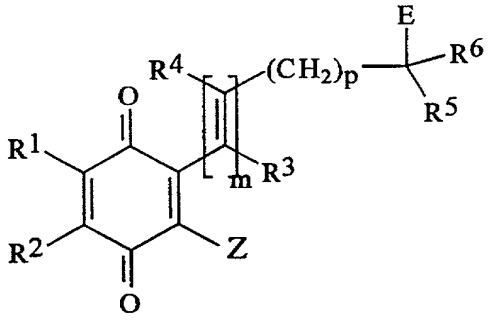
Following bioreduction of the conjugate and delivery of the drug species to the target site, the final form of the bioreductive carrier may itself comprise a stable, non-cytotoxic species, for example a compound having no potential alkylating centre. However, in a preferred embodiment of the invention, cytotoxicity of the bioreductive moiety may be reduced by providing a nucleophilic centre within the bioreductive compound itself. Following release of the drug an alkylating centre is formed. However, the proximity of the nucleophilic centre ensures that intramolecular alkylation occurs in preference to alkylation of any biomolecules such as DNA. In this way, substantially no cytotoxic species are formed. Such systems may be referred to as "self-alkylating".

Examples of electron rich groups capable of acting as a nucleophilic moiety in the bioreductive compound include oxygen, sulphur and nitrogen atoms. Thus, for example, inclusion of a suitably positioned amino, thio or hydroxyl group within the bioreductive compound will favour intramolecular alkylation resulting in a non-cytotoxic product on release of the drug at the site of hypoxia/ischemia. Suitable nucleophilic moieties which may be present in the bioreductive moiety include -OH, -SH, $-NH_2$ and -NHR in which R is C_{1-6} alkyl, e.g. C_{1-3} alkyl. Other suitable nucleophilic moieties will be known to those skilled in the art.

Alternatively, the bioreductive compound for use in the invention may be rendered non-cytotoxic following drug delivery by means of the introduction of steric hindrance capable of presenting a physical blockage to attack upon the bioreductive by any nucleophile. Thus, the presence of a bulky group either at or in close proximity to any potential alkylating centre generated

in the bioreductive moiety following drug delivery serves to abolish alkylating reactivity thus preventing alkylation of any biomolecules. Examples of groups which may be used in this way include linear or, more preferably, branched, C_{4-20} alkyl or alkenyl groups, e.g. tert. butyl. Other groups capable of providing steric hindrance will be known to those skilled in the art.

Particularly preferred bioreductive conjugates in accordance with the invention include compounds of formula II:



(II)

(wherein

R^1 and R^2 independently represent hydrogen or halogen atoms, or a group R, OR, SR, NHR, NR_2, CO_2R or CONHR;

or, alternatively, R^1 and R^2 together with the intervening ring carbon atoms form a 5-7 membered, preferably 5- or 6-membered, carbocyclic or heterocyclic ring itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

Z represents an alkyl, alkenyl, aryl or aralkyl group optionally carrying at least one OH, SH, NH_2 or NHR^7 group in which R^7 is an alkyl group;

R^3, R^4, R^5 and R^6 independently represent hydrogen atoms

or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L;

m = 0, 1, 2 or 3, preferably 1;

p = 0 or 2, preferably 0;

with the proviso that when m = 1 then p = 0)

or a salt thereof.

Preferred compounds of formula II include those wherein Z represents a group of the formula $(CH_2)_nXH$ in which n = 0, 1, 2 or 3, preferably 0; and X represents an oxygen or sulphur atom or, preferably, X represents a group of formula NY wherein Y represents a hydrogen atom or an alkyl group. Such compounds may act as "self-alkylating" systems.

Particularly preferred compounds of formula II are those wherein Z represents a group of the formula $(CH_2)_nXH$ in which X represents an amino group;

R^1 and R^2 each represent alkoxy groups or, together with the intervening ring carbon atoms, R^1 and R^2 form a benzene ring;

R^3, R^4, R^5 and R^6 each represent hydrogen atoms; and

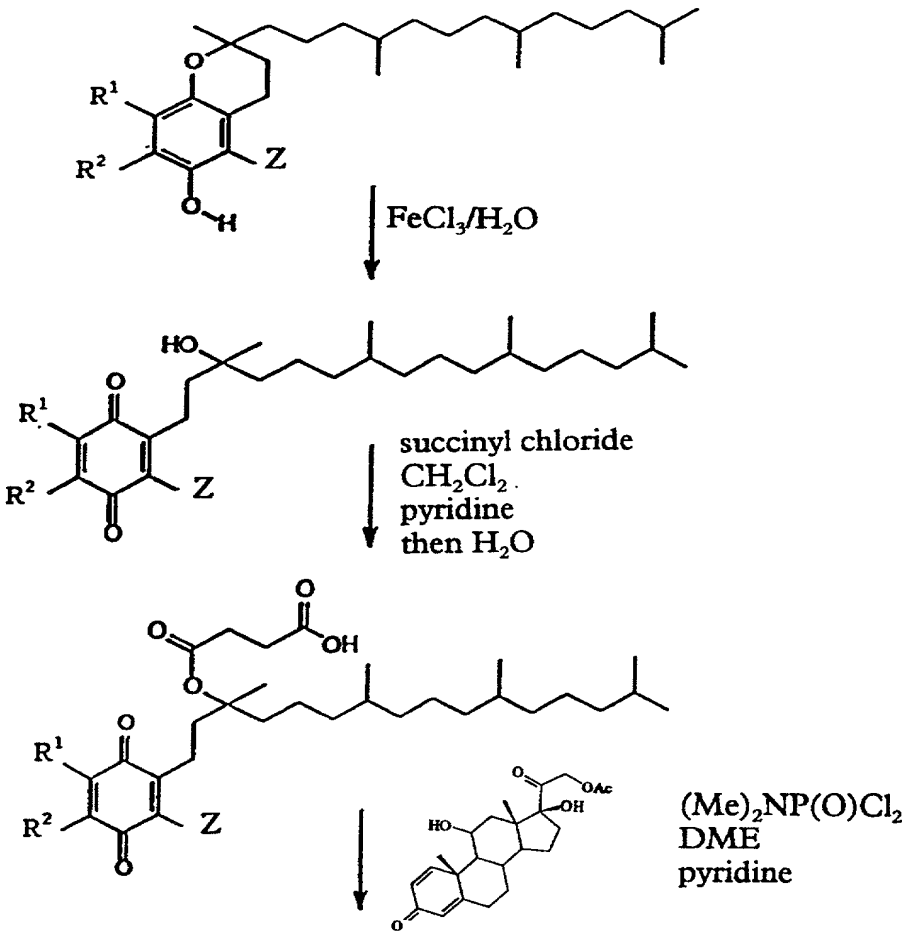
n = 0, m = 1 and p = 0.

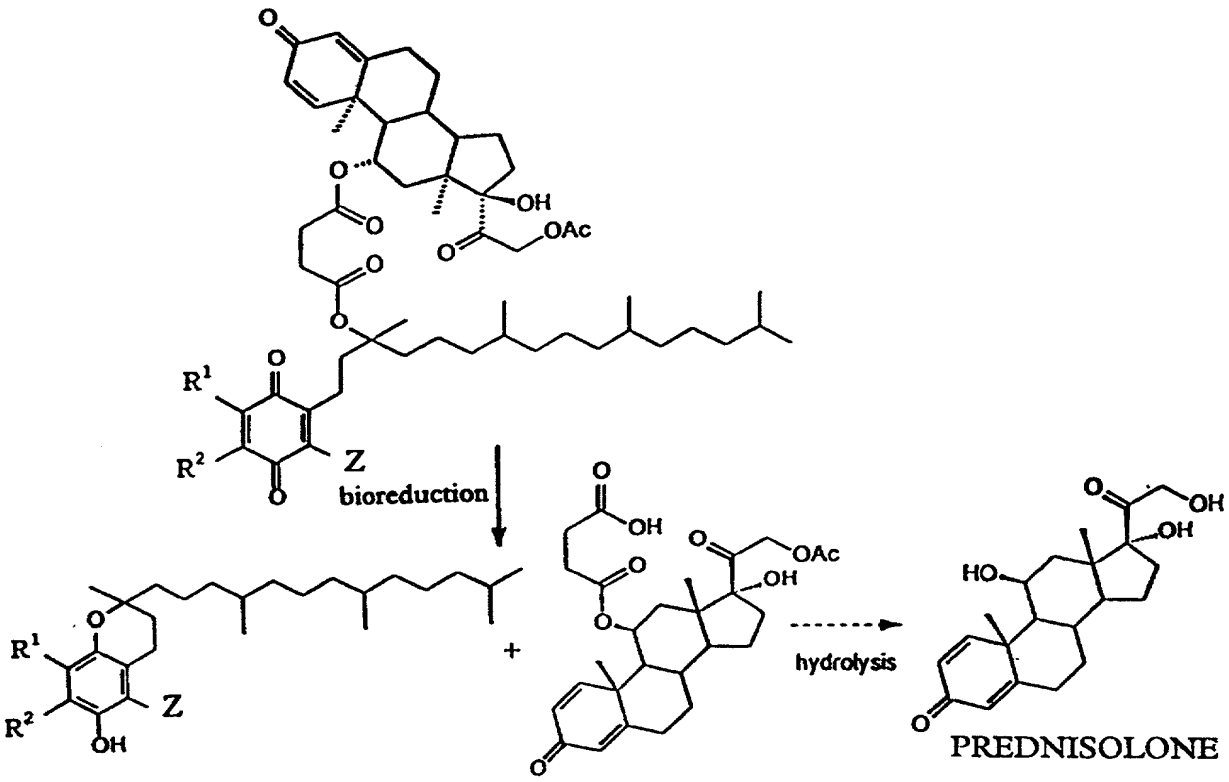
Alternatively, in relation to the compounds of

formula II, particularly when Z is other than a group of the formula $(CH_2)_nXH$, reduction of the quinone to its hydroquinone form may facilitate an intramolecular cyclisation reaction via the hydroxy group present on the hydroquinone ring and subsequent elimination of the drug species. The resulting cyclic ether is non-cytotoxic.

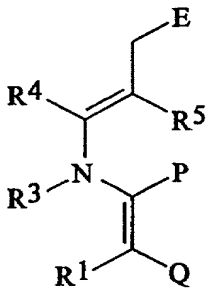
Reaction scheme 1 below illustrates the preparation of a preferred bioreductive conjugate of formula II in which R^1, R^2 and Z are as hereinbefore defined. As will be seen, bioreductive activation of the conjugate results in the formation of a cyclic ether which is an analogue of vitamin E and non-cytotoxic.

Scheme 1:





Other preferred bioreductive conjugates in accordance with the invention include those compounds of formula III:



(III)

(wherein

P and Q together with the intervening ring carbon atoms form a quinone or indoloquinone ring, a nitroaromatic, N-oxide or diazoaromatic compound, itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

R^1 represents a hydrogen or halogen atom, or a group R, OR, SR, NHR, NR_2, CO_2R or CONHR;

R^3, R^4 and R^5 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L);

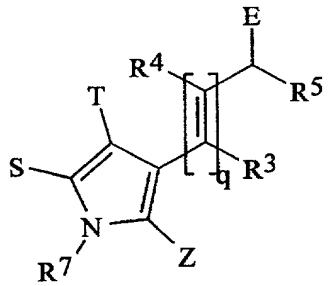
or a salt thereof.

Preferred compounds of formula III are those wherein P and Q together with the intervening ring carbon atoms form a quinone or indoloquinone ring; and

R^1, R^3, R^4 and R^5 each represent hydrogen atoms or methyl groups.

To act as "self-alkylating" systems, the electron-rich heteroatom present in the reduced form of the ring system of the compounds of formula III should preferably be no more than 6 bonds from the carbon atom linked to the therapeutic agent, E.

Other preferred bioreductive conjugates in accordance with the invention include the compounds of formula IV:



(IV)

(wherein

S and T together with the intervening ring carbon atoms form a quinone or iminoquinone ring, a nitroaromatic or N-oxide, e.g. an aromatic N-oxide, compound, itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

Z represents an alkyl, alkenyl, aryl or aralkyl group optionally carrying at least one OH, SH, NH_2 or NHR^6 group in which R^6 is an alkyl group;

R^7 represents an alkyl group, preferably C_{1-2} alkyl;

R^3, R^4 and R^5 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

q = 0, 1, 2 or 3, preferably 0 or 1;

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L);

or a salt thereof.

Preferred compounds of formula IV are those in which S and T together with the intervening ring carbon atoms form a quinone or N-oxide compound;

R^3, R^4 and R^5 each represent hydrogen atoms;

R^7 is methyl;

Z represents a group of formula $(CH_2)_nXH$ wherein X

represents an oxygen or sulphur atom or, preferably, a group of formula NY in which Y represents a hydrogen atom or an alkyl group, and n = 0, 1, 2 or 3; and

q = 0 or 1.

In relation to the compounds of formula IV, alkylating activity may effectively be abolished following drug delivery by choosing as group Z a bulky group capable of providing steric hindrance. In such cases, Z is preferably a linear or, more preferably, branched, C_{4-20} alkyl or alkenyl group. Alternatively, such compounds may act as "self-alkylating" systems in cases where Z represents a group of the formula $(CH_2)_nXH$.

In each of the compounds of general formulae II-IV above, the substituents R, R^1, R^2, R^3, R^4, R^5, R^6 and R^7 may be selected to provide the conjugate with optimum redox potential, solubility, enzyme specificity etc.

As used herein, the term "heterocyclic group" is intended to define a carbocyclic group interrupted by at least one heteroatom selected from oxygen, sulphur and nitrogen.

Examples of preferred carbocyclic or heterocyclic rings include benzene, pyridine, pyrrole, furan, pyrazine, piperidine, piperazine, pyrrolidine, morpholine and thiomorpholine rings.

In each of the compounds of formulae II-IV, preferred halogen atoms are fluorine and chlorine.

In the bioreductive conjugates of the invention, any alkyl or alkenyl moiety, unless otherwise stated, may be straight-chained or branched and preferably contains from 1 to 8, more preferably 1 to 6, and especially preferably 1 to 4, carbon atoms. Aryl moieties, unless otherwise stated, preferably contain from 5 to 12 ring atoms and especially preferably comprise phenyl rings.

Preferred salts of the compounds of formulae I-IV

are those which are suitable for administration to patients and are thus pharmaceutically or physiologically acceptable salts. Such salts may be formed with various inorganic and organic acids and include the ammonium, alkali and alkaline earth metal salts.

Reductases known to be involved in activation of bioreductive compounds include DT diaphorase, cytochrome P450, NADPH-dependent cytochrome P450 reductase and xanthine oxidase. The ease of reduction of any given bioreductive agent will depend upon its ability to act as a substrate for the intracellular reductases and the expression levels of such enzymes within the particular cell type. The choice of bioreductive compound for use in the invention will thus depend upon the type of enzymes present at the target site. Indeed, it may be useful to determine the relative enzyme activities in the target tissues of individual patients before starting treatment.

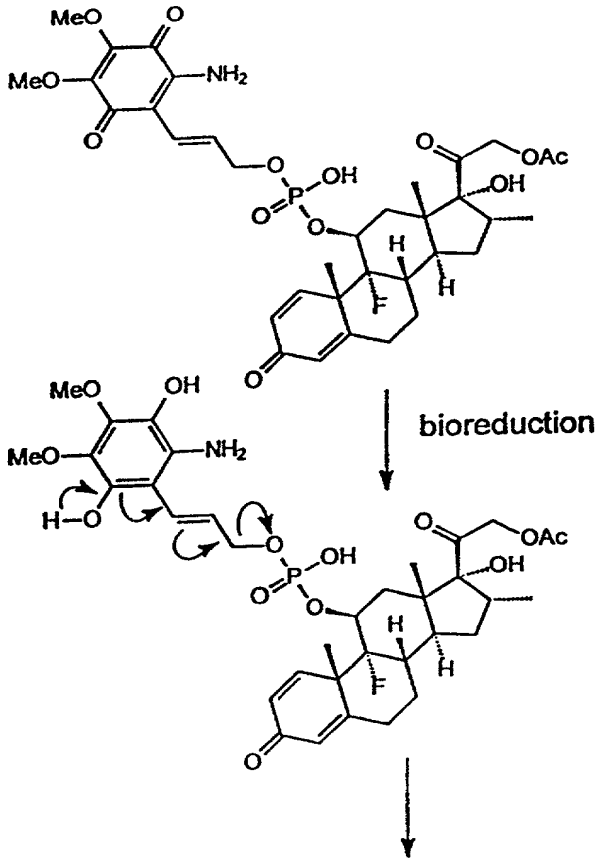
It is clearly desirable that the bioreductive conjugate should reach the target site intact. Since bioreduction of the conjugate is dependent upon the redox potential of the bioreductive moiety present, this may be selected such that this is less susceptible to reduction by ubiquitous systems such as NADH or NADPH, thereby increasing the chances that the conjugate will reach the target site still intact. In general, those bioreductive compounds having an optimal redox potential will be more selective in targeting of hypoxic cells and are thus preferred for use in the invention.

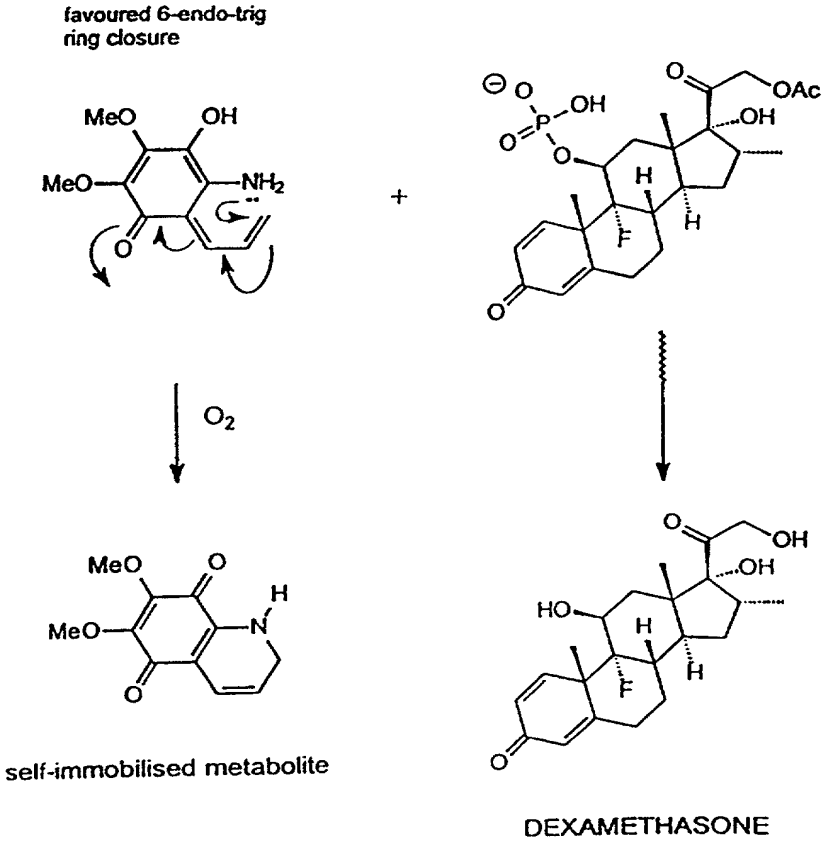
Examples of bioreductive compounds preferred for use in the invention include the quinones, naphthoquinones, indoloquinones and quinolino quinones and their derivatives. The electron deficient quinone nucleus in such compounds readily undergoes reduction *in vivo* to form the corresponding electron rich hydroquinone which in turn is capable of intramolecular

rearrangement to release the drug. Particularly preferred quinones include the 1,4-benzoquinones and the naphthoquinones in which the quinone ring carries an optionally hydroxy or amino substituted alkenyl group, e.g. a propenyl group, and an adjacent nucleophilic moiety, e.g. an amino group. Indoloquinones are particularly good substrates for DT diaphorase, an enzyme commonly found in most tissues.

A particularly preferred bioreductive conjugate in accordance with the invention is shown in reaction scheme 2 given below in which the bioreductive moiety is a 1,4-benzoquinone and the therapeutic agent is dexamethasone, an anti-inflammatory agent which may be used in the treatment of rheumatoid arthritis.

Scheme 2:





The invention is considered to have utility in connection with the delivery of a wide range of therapeutic agents. The expressions "therapeutic agent" and "drug" are used interchangeably herein and are intended to define any atom, ion or molecule which *in vivo* is capable of producing an effect detectable by any chemical, physical or biological examination. A therapeutic agent will in general be any substance which may be administered to a human or non-human animal body to produce a desired, usually beneficial, effect and may be an agent having either a therapeutic or a prophylactic effect.

Examples of therapeutic agents suitable for use in accordance with the invention include agents in all of the major therapeutic areas including anti-infectives such as antibiotics and antiviral agents, analgesics, anaesthetics and anti-inflammatory agents. Anti-

neoplastics, including known cytotoxic agents may also be used. The exact choice of therapeutic agent will naturally depend upon the desired therapeutic application.

Whilst it is envisaged that in general the therapeutic agent will itself be non-cytotoxic, the bioreductive carrier may be used to deliver cytotoxic agents, e.g. in anti-tumor treatment.

Examples of other therapeutic agents for use in acordance with the invention include agents administered to the human or animal body for diagnostic purposes, e.g. for use in radioimaging techniques. In this regard, a radiolabelled steroid may be linked to a non-cytotoxic bioreductive compound for use in the detection of hypoxic cells in tumor tissues.

Methods for attaching bioreductive compounds to a therapeutic agent are within the level of skill in the art. In general, the conjugates in accordance with the invention can be prepared by linkage of a non-cytotoxic bioreductive moiety to at least one therapeutic agent. Linkage of the therapeutic agent to the bioreductive moiety may be effected through any reactive group and standard coupling techniques are known in the art. Preferred reaction conditions, e.g. temperature, solvents, etc. depend primarily on the particular reactants and can readily be determined by those skilled in the art. In general, any reactive groups present, e.g. amino, carboxy etc. will be protected during coupling of the bioreductive with the therapeutic agent, although it is possible to leave some groups unprotected. After coupling, the resulting compound may be purified, e.g. by chromatography.

The bioreductive moiety may be bonded directly to the therapeutic agent or may be bonded by a linker group, L. Linkage between the bioreductive and the therapeutic agent may be effected via any reactive group present in the bioreductive moiety, e.g. a primary

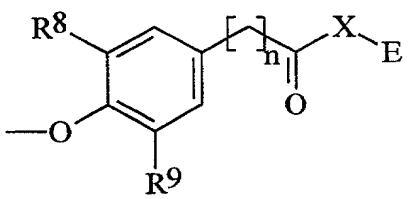
amine, carboxylate, alcohol, thiolate, etc. Preferably, the bioreductive moiety is linked to the therapeutic agent via an ester, phosphate ester, ether, amine, thiol or thiol ester bond or any combination thereof.

The linker group serves to link the bioreductive moiety to at least one therapeutic agent. Besides filling this role as a linker, the linker group may be selected to yield a bioreductive conjugate having desired characteristics. For example, appropriate choice of a linker group may serve to enhance the resistance of the conjugate to non-bioreductive metabolism and/or enhance delivery of the drug molecule at the target site. It may also be possible to optimise the redox potential, enzyme or tissue specificity, or the solubility of the conjugate by attaching to or incorporating within the linker group appropriately selected moieties, e.g. groups which are tissue targeting. Thus, the ability to alter the nature of the linker group provides for the possibility of altering the physicochemical properties, e.g. solubility, and biological properties, e.g. biodistribution, of the bioreductive conjugate. The primary function of the linker is however to link together the bioreductive compound and the drug.

Linker groups L particularly suitable for use in the invention for those drugs having a free -OH or -SH group include the following in which E represents the residue of a drug species:

$$-O-CO-(CH_2)_n-CO-X-E$$

and



(wherein n is an integer from 1 to 3;

X represents a sulphur or oxygen atom which may form part of the drug molecule E;

and R^8 and R^9 each independently represent F or Cl).

The bioreductive itself may be synthesised in accordance with conventional synthesis techniques. Techniques for the synthesis of quinones, in particular indoloquinones are described for example in J. Org. Chem. 50:4276-4281 (1985).

Viewed from a further aspect the invention provides a process for the preparation of a bioreductive conjugate comprising a non-cytotoxic bioreductive moiety with linked thereto at least one therapeutic agent, said process comprising linking at least one therapeutic agent to a non-cytotoxic bioreductive moiety.

There are believed to be many conditions which may benefit from the drug delivery system of the invention. These are primarily conditions associated with hypoxia and/or ischemia. Hypoxia is any state in which a physiologically inadequate amount of oxygen is available to, or utilised by, any given tissue or group of tissues within the body. Ischemia is any local diminution in the blood supply to any tissue in the body and may arise as a result of obstruction in the flow of arterial blood or vasoconstriction. In general, ischemia will ultimately lead to hypoxia.

In a clinical setting, tissues may become hypoxic and/or ischemic as a result of a number of different conditions in the body. Reduction of the blood supply to body tissues has the effect of inducing ischemia, for example in atherosclerosis, diabetes or following tissue or organ transplantation. Inflammatory or cancerous response may also lead to the tissue either physically

or metabolically outgrowing its vascular supply, again leading to ischemia and/or hypoxia.

Non-limiting examples of conditions which may be treated using the bioreductive conjugates of the invention include inflammatory conditions, e.g. rheumatoid arthritis, and other arthritic conditions such as osteoarthritis, diabetes, atherosclerosis, stroke, sepsis, Alzheimer's disease and other neurological diseases, cancer, kidney disease, digestive diseases and liver disease. Other conditions of interest include chronic periodontitis and ischemia following tissue transplantation.

The bioreductive conjugates of the invention may also find use in the treatment of a wide range of inflammatory conditions in which hypoxia and/or ischemia may be implicated, in particular in treating inflammatory conditions of the soft tissues. In the case of certain inflammatory conditions of the gastrointestinal tract, sections of the g.i. tract become hypoxic. Other inflammatory conditions which may be treated in accordance with the invention thus include gastrointestinal disorders such as Crohn's disease.

The compounds of the invention may also be used in the treatment of muscular disorders associated with hypoxia and/or ischemia.

It is believed that many known drugs could have enhanced therapeutic effects if selectively delivered to ischemic/hypoxic tissue. For example, following a cerebral attack, cerebral perfusion is reduced and the brain suffers an inflammatory response. The linkage of a vasodilator, such as a nitric oxide generator, or an anti-inflammatory agent, such as a steroid, to a bioreductive agent would thus serve to enhance the therapeutic index of the drug.

Rheumatoid arthritis is known to be associated with chronic synovial inflammation and poor perfusion of the synovial tissues. However, we have now discovered that

in patients suffering from rheumatoid arthritis the synovial tissues are in many cases profoundly hypoxic (pO_2 < 12 mm Hg). We have also found that such tissues contain high levels of reductases. Whilst not wishing to be bound by theoretical considerations, it is believed that there are pockets in the synovium which are hypoxic and that it is the hypoxic cells in the synovium which are primarily responsible for the inflammation associated with rheumatoid arthritis. Linkage of an anti-inflammatory agent, such as a non-steroidal anti-inflammatory agent, e.g. dexamethasone, a steroid or a nitric oxide inhibitor would thus serve to greatly increase the therapeutic index of the active agent in the treatment of rheumatoid arthritis, whilst at the same time reducing the risk of systemic side effects. The weak acidic based NSAIDs which undergo ion-trapping in acidotic tissue are considered particularly suitable.

Following transplantation and tissue rejection, both ischemia and an immunological-inflammatory response may contribute to tissue hypoxia. Again, such conditions may thus be treated using a conjugate of the invention in which a bioreductive moiety is linked to a vasodilator or to an anti-inflammatory or immunological suppressant.

Many of the basic complications of diabetes are believed to owe their basic pathology to hypoxia. Indeed, in many cases diabetics show accelerated atherosclerosis. The present invention may thus be used in the treatment of diabetes by linking a drug, such as a phosphodiesterase inhibitor, to a non-cytotoxic bioreductive moiety.

Hypoxic tissues are also believed to be present in chronic periodontitis, a condition associated with severe inflammation of the periodontium. Linkage of an antibiotic or other drug known for treating periodontitis, e.g. a metalloproteinase inhibitor, to a

bioreductive may thus be beneficial in treating this condition.

An example of an agent which may be linked to a non-cytotoxic bioreductive compound for use in treating diabetes is dipyridamole.

Viewed from a yet further aspect, the invention provides a bioreductive conjugate as hereinbefore defined for use in a method of targeting a therapeutic agent to a specific tissue site within the body, in particular to a site of hypoxia and/or ischemia, e.g. in the treatment of rheumatoid arthritis or other arthritic conditions, diabetes, atherosclerosis, stroke, sepsis, Alzheimer's disease and other neurological disorders, cancer, kidney disease, digestive diseases, liver disease, chronic periodontitis or ischemia following tissue transplantation.

In a preferred embodiment the invention provides a bioreductive conjugate comprising a non-cytotoxic bioreductive moiety linked to an anti-inflammatory agent for use in the treatment of rheumatoid arthritis.

Viewed from a yet further aspect the invention provides the use of a bioreductive conjugate as hereinbefore defined in the manufacture of a medicament for use as a targeting agent, in particular as an agent capable of targeting a site of hypoxia and/or ischemia within the body, e.g. in the treatment of rheumatoid arthritis and other arthritic conditions, diabetes, atherosclerosis, stroke, sepsis, Alzheimer's disease and other neurological disorders, cancer, kidney disease, digestive diseases, liver disease, chronic periodontitis or ischemia following tissue transplantation.

In another aspect the invention provides a method of targeting hypoxic and/or ischemic tissues in the human or non-human, preferably mammalian, body comprising administering to said body a bioreductive conjugate as hereinbefore defined. In particular, the invention provides a method of treating or preventing

rheumatoid arthritis and other arthritic conditions, diabetes, atherosclerosis, stroke, sepsis, Alzheimer's disease and other neurological disorders, cancer, kidney disease, digestive diseases, liver disease, chronic peridontitis or ischemia following tissue transplantation, said method comprising administering to a human or non-human animal body in need thereof an effective amount of a bioreductive conjugate as hereinbefore defined.

Viewed from a yet further aspect the invention provides a pharmaceutical composition comprising a bioreductive conjugate in accordance with the invention or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutical carrier or excipient.

The active ingredient in such compositions may comprise from about 0.1% to about 99% by weight of the formulation. By "pharmaceutically acceptable" is meant that the ingredient must be compatible with other ingredients of the compositions as well as physiologically acceptable to the patient.

Pharmaceutical compositions for use according to the present invention may be formulated in conventional manner using readily available pharmaceutical or veterinary aids. Thus the active ingredient may be incorporated, optionally together with other active substances, with one or more conventional carriers, diluents and/or excipients, to produce conventional galenic preparations such as tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, soft and hard gelatin capsules, suppositories, sterile injectable solutions, sterile packaged powders, and the like.

Examples of suitable carriers, excipients, and diluents are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, aglinates, tragacanth, gelatin, calcium silicate,

microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, water, water/ethanol, water/gylcol, water/polyethylene, glycol, propylene glycol, methyl cellulose, methylhydroxybenzoates, propyl hydroxybenzoates, talc, magnesium stearate, mineral oil or fatty substances such as hard fat or suitable mixtures thereof. The compositions may additionally include lubricating agents, wetting agents, emulsifying agents, suspending agents, preserving agents, sweetening agents, flavouring agents, and the like. The formulations may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by use of procedures well known in the art.

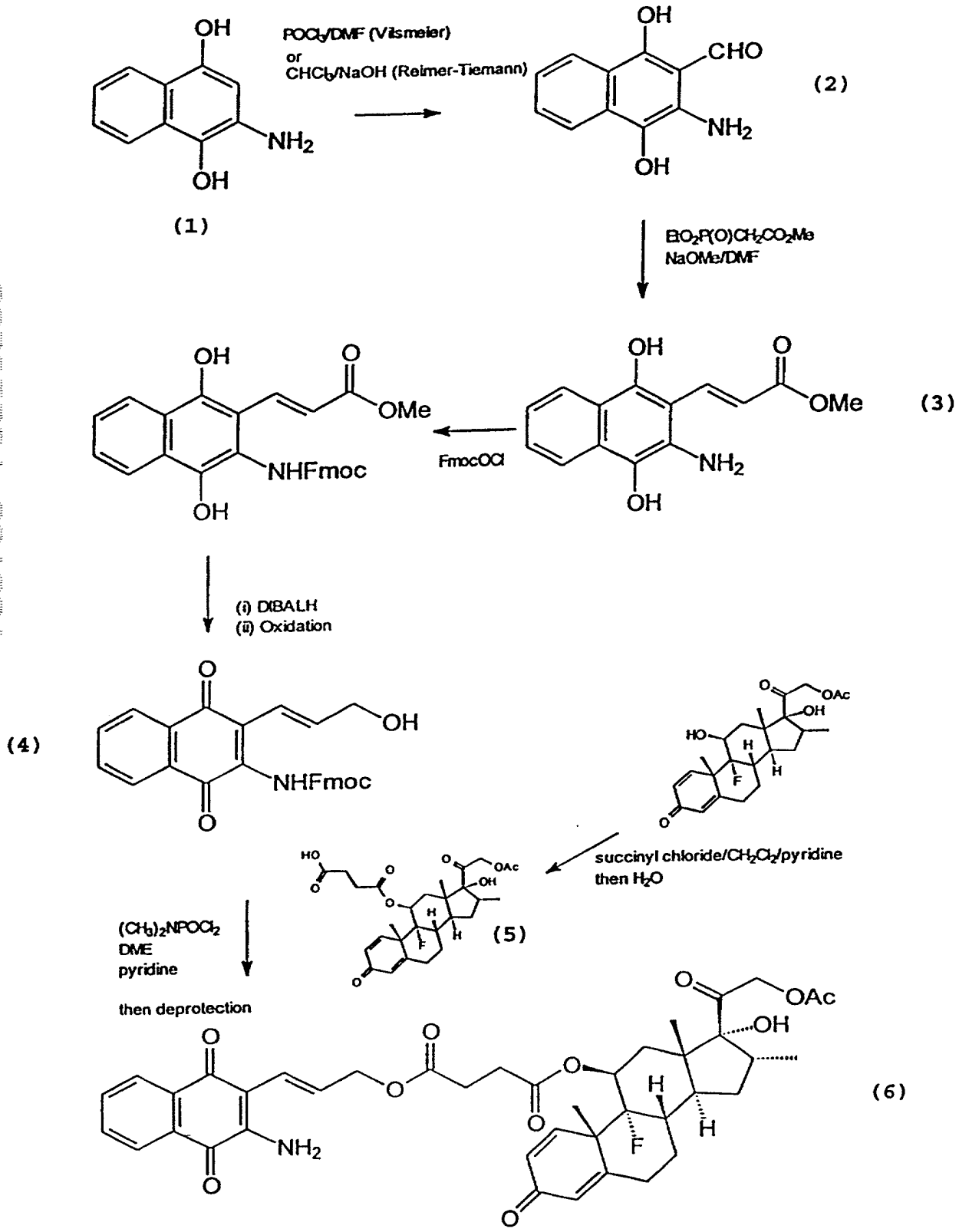
The compositions are preferably formulated in a unit dosage form, e.g. with each dosage containing from about 0.1 to about 500mg of the active ingredient.

The precise dosage of the active ingredient and the length of the treatment will depend upon a number of factors including the age and weight of the patient, the specific condition being treated and its severity, and the route of administration. In general, an effective dose will be of the order of from about 0.01 mg/kg to about 20 mg/kg bodyweight per day, e.g. from about 0.05 to about 10 mg/kg per day, administered one or more times daily. Thus, an appropriate dose for an adult may be from 10 to 100 mg per day, e.g. 20 to 50 mg per day.

Administration may be by any suitable method known in the art, including for example oral, parenteral (e.g. intramuscular, subcutaneous, intraperitoneal or intravenous), rectal or topical administration.

The present invention will now be further illustrated by way of the following non-limiting Examples and with reference to accompanying Figure 1 which shows the product profile obtained on the reduction of the aspirin-bioreductive conjugate of Example 5 by the $(CH_3)_2C^{\bullet}OH$ radical.

Example 1 - Synthesis of "self-alkylating" bioreductive delivery system.



Step 1 - N,N-dimethyl formamide (2 equivs) and $POCl_3$ are stirred together. The resulting solution is then added to a solution of the protected amino-dihydro-napthoquinone (1 equiv) in 1,2-dichloroethane and heated under reflux for about 1½ hours. The resulting solution is then cooled and NaOAc (1M, 100 mL/g quinone) is added with stirring over 2½ hours. The solution is then extracted with EtOAc, dried and evaporated. The resulting product **(2)** is then purified by chromatography on silica.

Step 2 - triethylphosphonoacetate (10.92 mmol) is stirred into dimethylformamide (80 ml). NaOMe (11 mmol) is then added and the solution is stirred for ½ hour. Product **(2)** (4.27 mmol) dissolved in dimethylformamide (20 ml) is added stepwise and stirring is continued for a further 2 hours. The mixture is then diluted with ethyl acetate (300 mL), washed with aqueous sodium hydrogen carbonate (6 x 100 mL), dried, evaporated *in vacuo* and the product **(3)** is recrystallised from ethyl acetate.

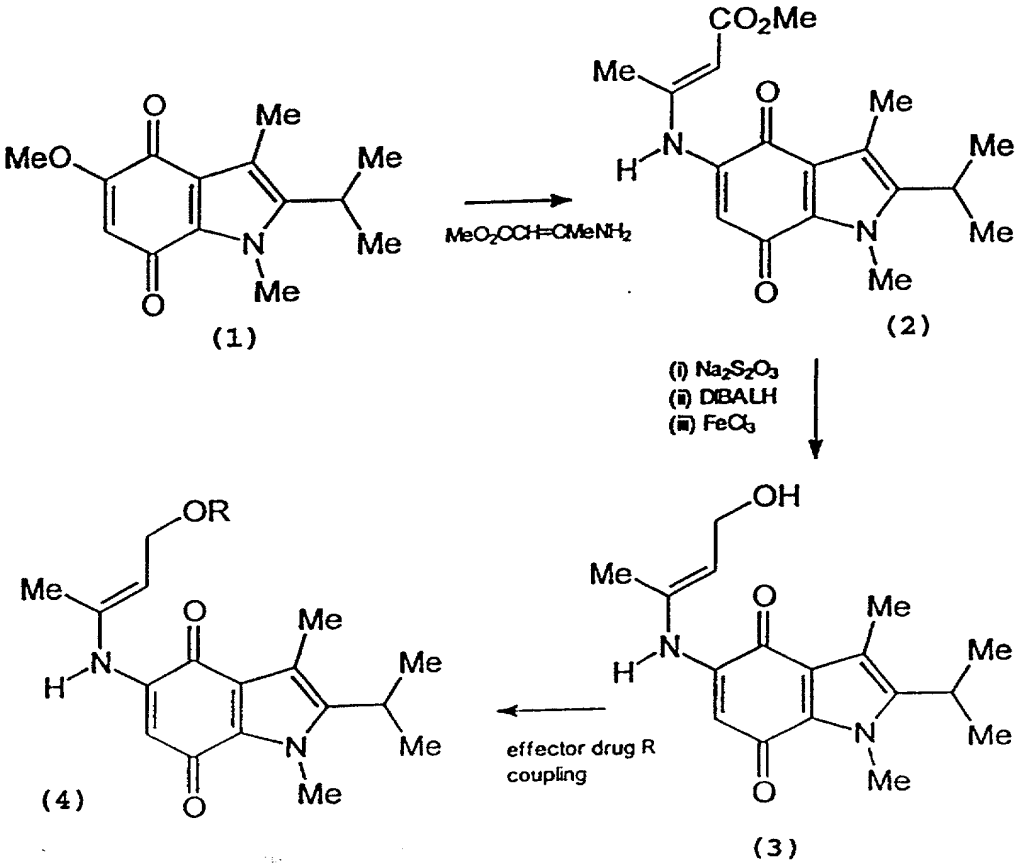
Step 3 - Product **(3)** (1.21 mmol) is dissolved in anhydrous CH_2Cl_2 (90 mL) and diisobutylaluminium hydride (16.3 mL, 1.5M in toluene) is added dropwise at -50°C. The mixture is then stirred for 3½ hours at -30°C and $FeCl_3$ (1.0M dissolved in 0.1M HCl, 27 mL) is added keeping the temperature below 0°C. Stirring is continued for a further ½ hour at 0°C followed by filtration. The resulting product is extracted with $CHCl_3$ (4 x 75 mL), washed with brine (50 mL), dried and evaporated *in vacuo*. Product **(4)** is recrystallised in ethanol.

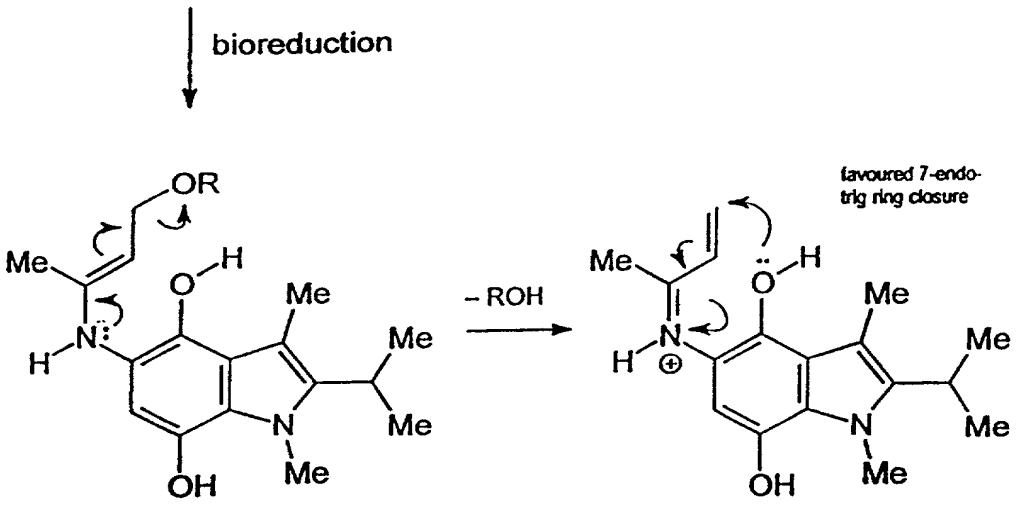
Step 4 - prednisolene 21-acetate (1 equiv) is dissolved in dry CH_2Cl_2 (50 mL) and dry pyridine (10 mL) is added under an atmosphere of nitrogen. The solution is then

stirred under reflux for 2 hours together with succinyl chloride (1.1 equivs). This is then cooled and washed with dilute HCl (0.1M, 20 mL) followed by H_2O (3 x 30 mL), dried and evaporated *in vacuo*. Product **(5)** is purified by chromatography on silica.

Step 5 - pyridine (6 mmol), N,N'-dimethylphosphoramidic dichloride (3 mmol) and product **(4)** (4 mmol) are added to a solution of product **(5)** (2 mmol) in 1,2-dimethoxyethane (10 mL) at 0°C. The resulting solution is stirred at ambient temperature under an atmosphere of argon for 16 hours. This is then poured into ice cold 1N HCl (40 mL) and extracted with CH_2Cl_2 (4 x 30 mL). The combined extracts are dried with $MgSO_4$, filtered and concentrated. The residue is purified by column chromatography on silica gel to give the final product **(6)**.

Example 2 - Synthesis of "self-alkylating" bioreductive delivery system.





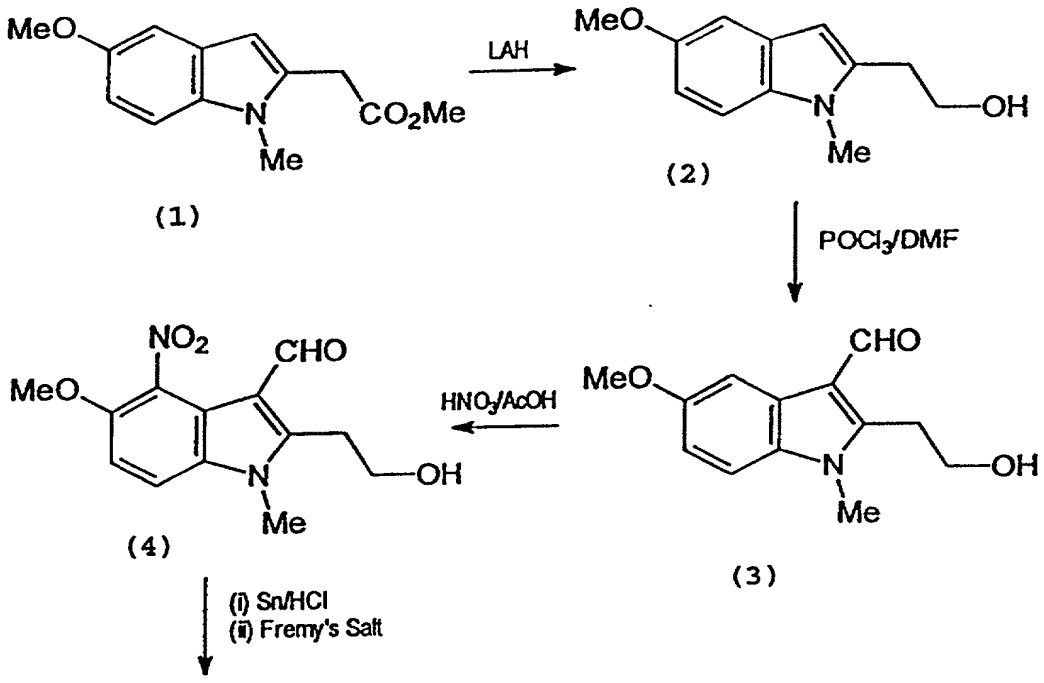
Step 1 - Compound **(1)** (10 mmol) (see Naylor et al., 2-Cyclopropyl Indoloquinones and their Analogues As Bioreductively-Activated Antitumor Agents: Structure-Activity *in vitro* and Efficacy *in vivo*, J. Med. Chem.: 40(15), 1997) is dissolved in DMF (10 mL) and methyl 3-aminocrotonate (50 mmol) is added. The reaction mixture is stirred at ambient temperature for 18 hours and then evaporated *in vacuo* and the residue purified on silica to give product **(2)**.

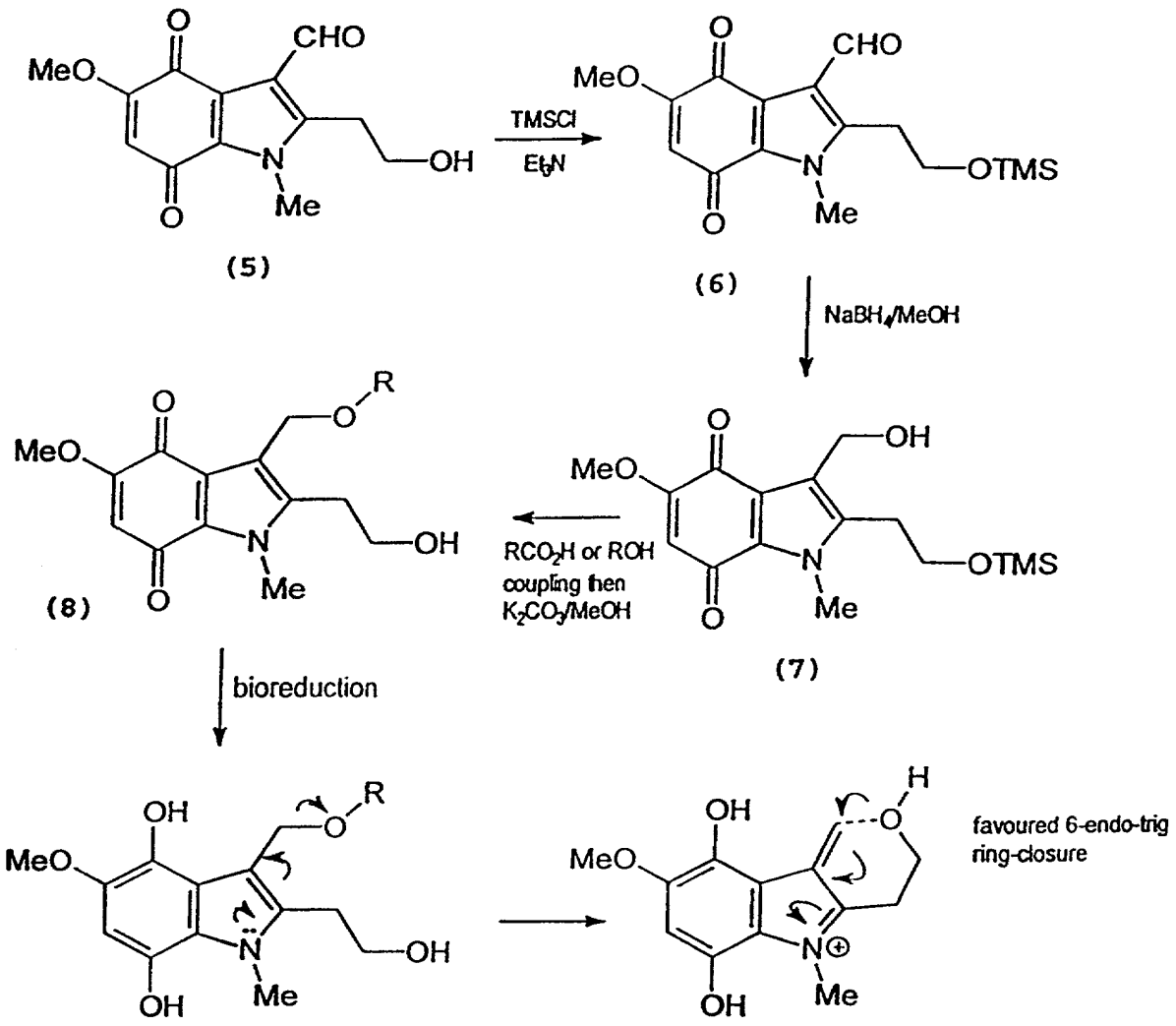
Step 2 - the aminocrotonate derivative **(2)** (10 mmol) is dissolved in $CHCl_3$ (300 mL) and EtOH (110 mL) and a solution of $Na_2S_2O_4$ (120 mmol) in H_2O (130 mL) added. The solution is stirred at ambient temperature for ½ hour and the organic layer separated, washed with saturated NaCl (500 mL), dried and evaporated. The crude hydroquinone is then dissolved in anhydrous CH_2Cl_2 (300 mL) under argon, cooled to -30°C and DIBAL-H (50 mL of a 1.5M solution in toluene) added dropwise such that the solution temperature remains below -30°C. The solution is then allowed to reach 0°C and stirred for 2½ hours at this temperature, and a solution of solution of $FeCl_3$ (90 mL, 1.0M (0.1M HCl)) added. The solution is stirred for 10 min at 0°C and then $CHCl_3$ (500 mL) and H_2O

(500 mL) added. The aqueous layer is extracted with $CHCl_3$ (5 x 250 mL) then EtOAc (5 x 250 mL) and the combined organic phases washed with saturated NaCl (500 mL), dried and evaporated. The residue is purified on silica and recrystallized from EtOAc to give product **(3)** as a purple/red solid.

Step 3 - the indoloquinone **(3)** (10 mmol) is dissolved in THF (25 mL) and added to a solution (THF, 25 mL) of the drug carboxylic acid or phenol to be coupled (1.5 equivs), triphenylphosphine (20 mmol) and diethylazodicarboxylate (20 mmol). The solution is then stirred overnight at 50°C, the solvent evaporated and the residual final product **(4)** is purified on silica.

Example 3 - Synthesis of "self-alkylating" bioreductive delivery system.





Step 1 - Methyl 5-Methoxy-1-methylindole-2-acetate (10 mmol) is dissolved in anhydrous THF (250 mL) and $LiAlH_4$ (100 mL of a 1.0M solution in THF) added dropwise at ambient temperature and under argon. The solution is then stirred for 1 hour at 30°C and then EtOAc (250 mL) added, followed by the gradual addition of H_2O (150 mL). The solution is washed with HCl (0.1M, 250 mL) and saturated NaCl (250 mL), dried and evaporated. The residue is purified by flash column chromatography on silica and then recrystallized to give product **(2)**.

Step 2 - DMF (100 mmol) and $POCl_3$ (25 mmol) are stirred at -5°C for ½ hour and then a solution of **(2)** (10 mmol in 30 mL DMF) is added slowly, maintaining the temperature at about 0°C, and then warmed to 40°C and stirred for 1 hour. Ice/water (100 mL) is then added, followed by NaOH (37%, 50 mL) and the solution extracted into EtOAc, evaporated and the carboxaldehyde **(3)** purified by recrystallization from an EtOAc/hexane mixture.

Step 3 - to a solution of **(3)** (10 mmol) in AcOH (50 mL) cooled to 5°C, is added dropwise a cold (0°C) mixture of fuming HNO_3 (10 mL) in AcOH (30 mL). The solution is stirred for 1 hour while allowing to reach ambient temperature, and then poured onto 100g of crushed ice. After 15 minutes stirring the resulting yellow solid is collected by suction filtration. The dried residue is purified on silica to give product **(4)** as a yellow solid.

Step 4 - to a suspension of **(4)** (10 mmol) in EtOH (180 mL) is added tin powder (40 mmol) and HCl (3.0M, 70 mL) and the solution stirred at ambient temperature for 1 hour. The solution is then decanted from the excess tin and neutralized with saturated $NaHCO_3$(aq.). The resulting suspension is then added to an equal volume of H_2O and extracted with $CHCl_3$ (5 x 50 mL) and then EtOAc (5 x 50 mL) and the combined extracts evaporated. The residual 4-aminoindole derivative is purified on silica and used immediately in the next step by dissolving in Me_2CO (250 mL) and adding a solution of potassium nitrosodisulfonate ($(KSO_3)_2NO$, Fremy's salt, 30 mmol)) in NaH_2PO_4/Na_2HPO_4 buffer (250 mL, 0.3M, pH 6.0) and the solution stirred at ambient temperature for 1 hour. The Me_2CO is removed *in vacuo* and the resulting orange precipitate collected by suction filtration, washed with H_2O and dried in a vacuum oven at 45°C to

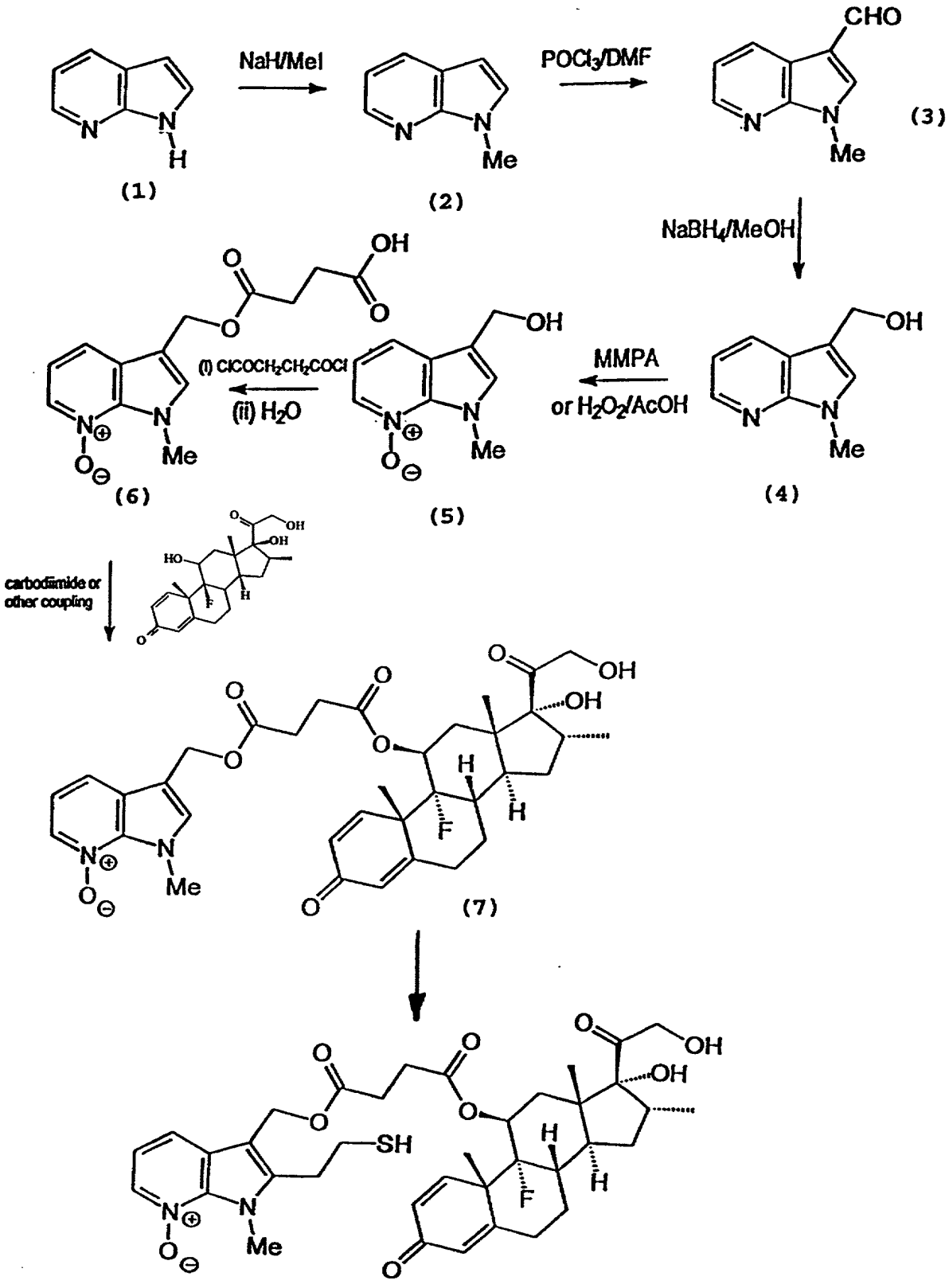
afford product **(5)** as an orange solid which is recrystallized from EtOAc.

Step 5 - indoloquinone **(5)** (10 mmol) is dissolved in THF (100 mL) together with Et_3N (10 mmol) and trimethylchlorosilane (1.1 mmol) added. The solution is stirred at ambient temperature for 8 hours, evaporated and purified on silica to give product **(6)**.

Step 6 - the protected indoloquinone **(6)** (10 mmol) is dissolved in anhydrous nitrogen degassed MeOH (200 mL) and $NaBH_4$ (30 mmol) added. The solution is degassed with argon and stirred for 5 min under argon and then aerated and diluted with EtOAc (700 mL) and washed with H_2O (2 x 250 mL) and then saturated NaCl (100 mL). The dried organic solution is condensed to give the indoloquinone **(7)** as an orange solid after silica column and/or recrystallization from EtOAc.

Step 7 - the 3-(hydroxymethyl)indoloquinone **(7)** is dissolved in THF (50 mL) together with triphenylphosphine (20 mmol) and diethylazodicarboxylate (20 mmol) and the desired drug carboxylic acid or phenol (RCO_2H or ROH where R is a drug species, 1.5 to 5 equivs) added. The solution is then stirred overnight at 50°C, the solvent evaporated and the residue redissolved in EtOAc. The solution is then washed with HCl (1.0M, 50 mL) and H_2O (50 mL), dried and evaporated. The product is purified on silica and deprotected by dissolving in anhydrous MeOH together with K_2CO_3 (10 mmol) at 0°C and stirring for 45 min. The final product **(8)** is then purified on silica and recrystallized from EtOAc.

Example 4 - Synthesis of "self-alkylating" bioreductive delivery system.



"SELF ALKYLATING" VERSION

Step 1 - 7-Azaindole (Sigma-Aldrich, 10 mmol) is added gradually with stirring to a suspension of NaH (11 mmol) in THF (30 mL). After 15 minutes, methyl iodide (10 mmol) is added and the solution stirred at ambient temperature for 1 hour. The solution is cooled to -5°C and H_2O (30 mL) added gradually, followed by EtOAc (50 mL). The aqueous layer is then further extracted with EtOAc (3 x 50 mL), washed with saturated $NaHCO_3$, saturated NaCl, dried and evaporated. The residue is purified on silica to give product **(2)**.

Step 2 - DMF (100 mmol) and $POCl_3$ (25 mmol) are stirred at -5°C for ½ hour and then a solution of **(2)** (10 mmol in 30 mL DMF) is added slowly, maintaining the temperature at about 0°C, and then warmed to 40°C and stirred for 1 hour. Ice/water (100 mL) is then added, followed by NaOH (37%, 50 mL) and the solution extracted into EtOAc, evaporated and the carboxaldehyde **(3)** purified by recrystallization from an EtOAc/hexane mixture.

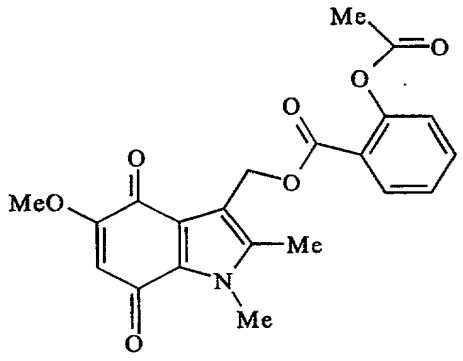
Step 3 - the 3-formyl-7-azaindole **(3)** (10 mmol) is dissolved in anhydrous nitrogen degassed MeOH (200 mL) and $NaBH_4$ (30 mmol) added. The solution is degassed with argon and stirred for 5 min under argon and then aerated and diluted with EtOAc (700 mL) and washed with H_2O (2 x 250 mL) and then saturated NaCl (100 mL). The dried organic solution is condensed to give the 3-hydroxymethyl derivative **(4)** after silica column chromatography.

Step 4 - product **(4)** (10 mmol) is dissolved in KOH (0.5M, aq., 100 mL). Caro's acid (potassium peroxymonosulphate, Oxone, $2KHSO_5.KHSO_4.K_2SO_4$, 10 mmol) is added slowly with stirring and the solution stirred for 12 hours. The solution is neutralised with phosphoric acid, evaporated and the residual salt extracted and purified on silica to afford **(5)**.

Step 5 - the 3-(hydroxymethyl)indole **(5)** (10 mmol) is dissolved in THF (50 mL) together with pyridine (5 mL) and succinylchloride (10 mmol) added with stirring. After 1 hour H_2O (50 mL) is added and the solution stirred for 1½ hours and 2.0M HCl (50 mL) added. After a further 1½ hours the solution is extracted with Et_2O (3 x 100 mL), dried and evaporated. The acid **(6)** is purified on silica.

Step 6 - the azaindole-N-oxide carboxylic acid **(6)** (10 mmol) is dissolved in THF (25 mL) and added to a solution (THF, 25 mL) of the protected steroid (1.5 equivs), triphenylphosphine (20 mmol) and diethylazodicarboxylate (20 mmol). The solution is then stirred overnight at 50°C, the solvent evaporated and the residue redissolved in EtOAc. The solution is washed with HCl (1.0M, 50 mL) and saturated $NaHCO_3$ (aq., 50 mL), dried and evaporated. The final product **(7)** is purified on silica.

Example 5 - Preparation of 3-(2-Acetoxybenzoyloxy) methyl-1,2-dimethyl-5-methoxyindole-4,7-dione: Aspirin-Bioreductive Conjugate



3-Hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione (0.235g, 1.0 mmol) was dissolved in dichloromethane (anhydrous, 25 mL) together with pyridine (2.5 mL). 2-Acetylsalicyloyl chloride (0.237g, 1.2 mmol) was then

added and the solution heated under reflux for 1½ hours, cooled and ethyl acetate (100 mL) added. The solution was washed with HCl (0.1 M, 100 mL) and then saturated NaCl (100 mL), dried and evaporated. The residue was purified on silica gel, eluting with ethyl acetate to afford the title compound as a yellow solid (275 mg, yield: 69.3%) which was recrystallised from ethyl acetate, mp 159-161°C.

^{1}H-NMR ($CDCl_3$) δ 2.27 (s, 3H), 2.31 (s,3H), 3.81 (s, 3H), 3.90 (s, 3H), 5.47 (s, 2H), 5.63 (s, 1H), 7.01-7.53 (m, 3H) and 7.99 (dd, J = 1.4 and 8.1 Hz, 1H) ppm.

Analysis: Found : C 63.81, H 4.81, N 3.71
Calculated : C 63.47, H 4.82, N 3.52%

Example 6

Pharmacokinetics of the indoloquinone-acetyl salicylic acid conjugate of Example 5 were studied as follows:

PROTOCOL:

Three groups of male Wistar albino rats (n=5) received sterile air dorsally (day 1). After two days a further 20 ml sterile air were administered. On day 5, 2 ml of a 1% carrageenin in sterile saline was injected directly into the air pouch. Animals were housed in metabolic cages.

100 mg of the indoloquinone-aspirin conjugate of Example 5 were suspended in ethanol (2 ml). 50 mg acetyl salicylic acid was dissolved in 2 ml ethanol. 2 ml ethanol was used as a control. 18 ml sterile water were added to each sample.

On day 9, each animal was injected with 4 ml of solution as follows:

Group A - 20 mg indoloquinone-aspirin conjugate
Group B - 10 mg acetyl salicylic acid
Group C - ethanol (control)

The animals were then returned to their cages for periods of either 2 (nos. 1, 2 and 3 from each group) or 4 hours (nos. 4 and 5 from each group). After this time the animals were anaesthetised and blood and exudate collected. Available urine was also collected.

RESULTS:

Analysis of the collected samples by HPLC showed that the bioreductive-acetyl salicylic acid conjugate had been cleaved to liberate acetyl salicylic acid.

Example 7

The reduction initiated release of aspirin from the indoloquinone-acetyl salicylic acid conjugate of Example 5 was investigated by product analysis (HPLC) following γ-radiolysis of N_2O-saturated solutions containing the quinone (100 μM) and 2-propanol (8.3M, 50%, v/v) at pH 7.4.

The radiation chemical yield (G) of the $(CH_3)_2C^{\bullet}OH$ radical in N_2O-saturated 2-propanol/water mixtures was determined by ferricyanide reduction to be $G((CH_3)_2C^{\bullet}OH) = 0.67 \pm 0.02$ μmol J^{-1} in 2-propanol/water (50%, v/v) and 0.72 ± 0.03 μmol J^{-1} in 1 M 2-propanol respectively. Figure 1 shows the product profile obtained on the reduction of the quinone by the $(CH_3)_2C^{\bullet}OH$ radical. Loss of the parent quinone ($G(-Q) = 1.63 \pm 0.01$ μmol J^{-1}) parallel the formation of the aspirin leaving group (LG) with $G(LG) = 1.40 \pm 0.15$ μmol J^{-1}.

The two remaining major peaks in Figure 1 were derived from the reaction of the resultant iminium

derivative with water to generate (a) and with the 2-propanol to generate the isopropyl ether (b). Both of these quinones are generated by autoxidation of their respective hydroquinones following the unavoidable introduction of oxygen during HPLC sampling:

$$QH_2 + O_2 \rightarrow Q + H_2O_2$$

As expected, the relative yields of (a) and (b) were dependent on the alcohol concentration, with the alkylation product virtually disappearing when radiolysis was performed in 1M 2-propanol.

Steady-state γ-radiolysis

Indolequinone solutions were saturated with N_2O gas in gas-tight vials before irradiation in a ^{60}Co source. An absorbed dose of 1 Gy = 0.67 μM $(CH_3)_2C^{\bullet}OH$ radicals in N_2O-saturated 2-propanol/water (50%, v/v). A dose rate of 6-6.5 Gy min^{-1} was used, as determined by Fricke dosimetry and radiation chemical yields were corrected for the absorbed dose in the various alcohol-water mixtures employed.

High performance liquid chromatography (HPLC)

Product analysis following γ-radiolysis was performed by gradient HPLC separation on a 100 mm x 4.6 mm base-deactivated reverse-phase column (Hichrom RPB, Hichrom, Reading, U.K.). The eluents were (A): KH_2PO_4 (5 mM), H_3PO_4 (5 mM), (B): CH_3CN/H_2O (3:1, v/v), with a flow rate of 2 cm^3 min^{-1}. One of two linear gradients was used for each compound: (1) 35-80% B in 8 min, or (2) 20-50% B in 5 min. Detection was at 232 nm using a Waters 486 detector (Watford, U.K.) and concentrations were determined from peak areas using Waters Maxima software.

Example 8 - Formulation

A composition suitable for use in the treatment of rheumatoid arthritis is produced using the following ingredients:

dexamethasone	5 mg
starch	45 mg
microcrystalline cellulose	35 mg
polyvinylpyrrolidone (as 10% solution in water)	4 mg
sodium carboxymethyl starch	4.5 mg
magnesium stearate	0.5 mg
talc	1 mg
total	95 mg

The active ingredient, starch and cellulose are sieved and mixed thoroughly. The aqueous solution contining polyvinylpyrrolidone is mixed with the resulting powder and the mixture is then passed through a sieve. The resulting granules are dried and sieved again. The sodium carboxymethyl starch, magnesium stearate and talc are sieved and then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets weighing 95 mg.

One tablet taken daily is suitable for the treatment of patients suffering from rheumatoid arthritis.

CLAIMS

1. A bioreductive conjugate comprising a non-cytotoxic bioreductive moiety with linked thereto at least one therapeutic agent, and salts thereof, said conjugate being such that on bioreduction the therapeutic agent is released with generation of a species having an alkylating centre and being capable of undergoing a self-alkylation reaction to generate a non-cytotoxic residue of the bioreductive moiety.

2. A bioreductive conjugate as claimed in claim 1 of formula I:

$$A\,(B)_n \qquad (I)$$

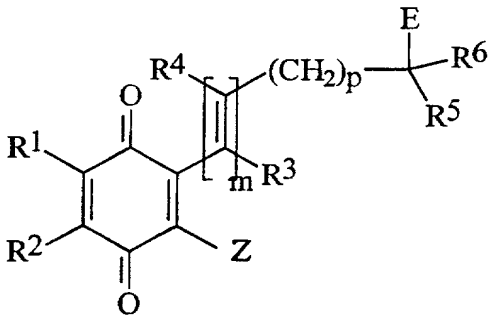
(where A is a non-cytotoxic bioreductive moiety, each B is independently the residue of a therapeutic agent, and n is an integer) or a salt thereof.

3. A bioreductive conjugate as claimed in claim 2, wherein in formula I, n is 1 to 3.

4. A bioreductive conjugate as claimed in claim 2 or claim 3, wherein A and B are stably conjugated in an oxygenated environment and are such that following reductive activation of A, A and B detach and either A is itself a stable, non-cytotoxic species, or A reacts with itself to form a stable, non-cytotoxic species.

5. A bioreductive conjugate as claimed in any one of claims 1 to 4, wherein said bioreductive moiety is substantially non-mutagenic.

6. A bioreductive conjugate as claimed in claim 1 of the formula II:



(II)

(wherein

R^1 and R^2 independently represent hydrogen or halogen atoms, or a group R, OR, SR, NHR, NR_2, CO_2R or CONHR;

or, alternatively, R^1 and R^2 together with the intervening ring carbon atoms form a 5-7 membered carbocyclic or heterocyclic ring itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

Z represents an alkyl, alkenyl, aryl or aralkyl group optionally carrying at least one OH, SH, NH_2 or NHR^7 group in which R^7 is an alkyl group;

R^3, R^4, R^5 and R^6 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L;

m = 0, 1, 2 or 3; and

p = 0 or 2;

with the proviso that when m = 1 then p = 0)

or a salt thereof.

7. A bioreductive conjugate as claimed in claim 6, wherein in formula II:

Z represents a group of the formula $(CH_2)_nXH$;

n = 0, 1, 2 or 3;

X represents an oxygen or sulphur atom, or a group of formula NY in which Y represents a hydrogen atom or an alkyl group;

or a salt thereof.

8. A bioreductive conjugate as claimed in claim 6, wherein in formula II:

Z represents a group of the formula $(CH_2)_nXH$ in which X represents an amino group;

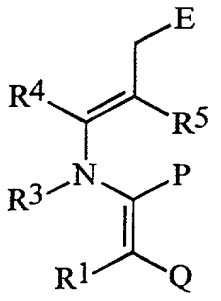
R^1 and R^2 each represent alkoxy groups or, together with the intervening ring carbon atoms, R^1 and R^2 form a benzene ring;

R^3, R^4, R^5 and R^6 each represent hydrogen atoms; and

n = 0, m = 1 and p = 0;

or a salt thereof.

9. A bioreductive conjugate as claimed in claim 1 of formula III:



(III)

(wherein

P and Q together with the intervening ring carbon atoms form a quinone or indoloquinone ring, a nitroaromatic, N-oxide or diazoaromatic compound, itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

R^1 represents a hydrogen or halogen atom, or a group R, OR, SR, NHR, NR_2, CO_2R or CONHR;

R^3, R^4 and R^5 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group; and

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L)

or a salt thereof.

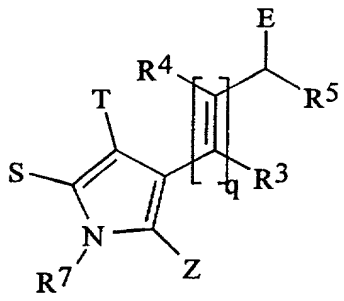
10. A bioreductive conjugate as claimed in claim 9, wherein in formula III:

P and Q together with the intervening ring carbon atoms form a quinone or indoloquinone ring; and

R^1, R^3, R^4 and R^5 each represent hydrogen atoms or methyl groups;

or a salt thereof.

11. A bioreductive conjugate as claimed in claim 1 of formula IV:



(IV)

(wherein

S and T together with the intervening ring carbon atoms form a quinone or iminoquinone ring, a nitroaromatic or N-oxide compound, itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

Z represents an alkyl, alkenyl, aryl or aralkyl group optionally carrying at least one OH, SH, NH_2 or NHR^6 group in which R^6 is an alkyl group;

R^7 represents an alkyl group;

R^3, R^4 and R^5 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

q = 0, 1, 2 or 3; and

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L)

or a salt thereof.

12. A bioreductive conjugate as claimed in claim 11, wherein in formula IV:

S and T together with the intervening ring carbon atoms form a quinone or N-oxide compound;

R^3, R^4 and R^5 each represent hydrogen atoms;

R^7 is methyl;

Z represents a group of formula $(CH_2)_nXH$ wherein X represents an oxygen or sulphur atom, or X represents a group of formula NY in which Y represents a hydrogen atom or an alkyl group; and

q = 0 or 1,

or a salt thereof.

13. A bioreductive conjugate as claimed in any one of claims 1 to 5, wherein said bioreductive moiety comprises a quinone, naphthoquinone, indoloquinone, quinolino quinone or a derivative thereof.

14. A bioreductive conjugate as claimed in claim 13, wherein said bioreductive moiety is a 1,4-benzoquinone, a naphthoquinone, or a derivative thereof, in which the quinone ring carries an optionally hydroxy- or amino-substituted alkenyl group and an adjacent nucleophilic moiety.

15. A bioreductive conjugate as claimed in any one of claims 1 to 5, wherein said bioreductive moiety is a

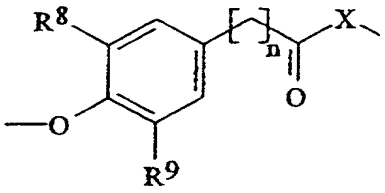
1,4-benzoquinone and the therapeutic agent is dexamethasone.

16. A bioreductive conjugate as claimed in any preceding claim, wherein said bioreductive moiety is linked to said therapeutic agent via a linker group L comprising an ester, phosphate ester, ether, amine, thiol or thiol ester group or any combination thereof.

17. A bioreductive conjugate as claimed in claim 15 wherein said linker group L is a group of the formula:

$$-O-CO-(CH_2)_n-CO-X-$$

or



(wherein n is an integer from 1 to 3;

X represents a sulphur or oxygen atom; and

R^8 and R^9 each independently represent F or Cl).

18. A bioreductive conjugate comprising a non-cytotoxic bioreductive noiety with linked thereto at least one therapeutic agent, and salts thereof, said conjugate being such that on bioreduction the therapeutic agent is released with generation of a species having a sterically hindered alkylating centre to prevent alkylation of biomolecules.

19. A process for the preparation of a bioreductive conjugate as claimed in any of claims 1 to 18, said process comprising linking at least one therapeutic agent to a non-cytotoxic bioreductive moiety.

20. A pharmaceutical composition comprising a bioreductive conjugate as claimed in any one of claims to to 18, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutical carrier or excipient.

21. A bioreductive conjugate as claimed in any one of claims 1 to 18 for use in a method of targeting a therapeutic agent to a site of hypoxia and/or ischemia within the human or non-human animal body.

22. A bioreductive conjugate as claimed in any one of claims 1 to 18 for use in treatment of rheumatoid arthritis or other arthritic conditions, diabetes, atherosclerosis, stroke, sepsis, Alzheimer's disease and other neurological disorders, cancer, kidney disease, digestive diseases, liver disease, chronic periodontitis or ischemia following tissue transplantation.

23. Use of a bioreductive conjugate as claimed in any one of claims 1 to 18 in the manufacture of a medicament for use as a targeting agent capable of targeting a site of hypoxia and/or ischemia within the human or non-human animal body.

24. Use as claimed in claim 22 for the treatment of rheumatoid arthritis or other arthritic conditions, diabetes, atherosclerosis, stroke, sepsis, alzheimer's disease and

other neurological disorders, cancer, kidney disease, digestive diseases, liver disease, chronic periodontitis or ischemia following tissue transplantation.

25. A method of targeting hypoxic and/or ischemic tissues in the human or non-human animal body, said method comprising administering to said body a bioreductive conjugate as claimed in any one of claims 1 to 18.

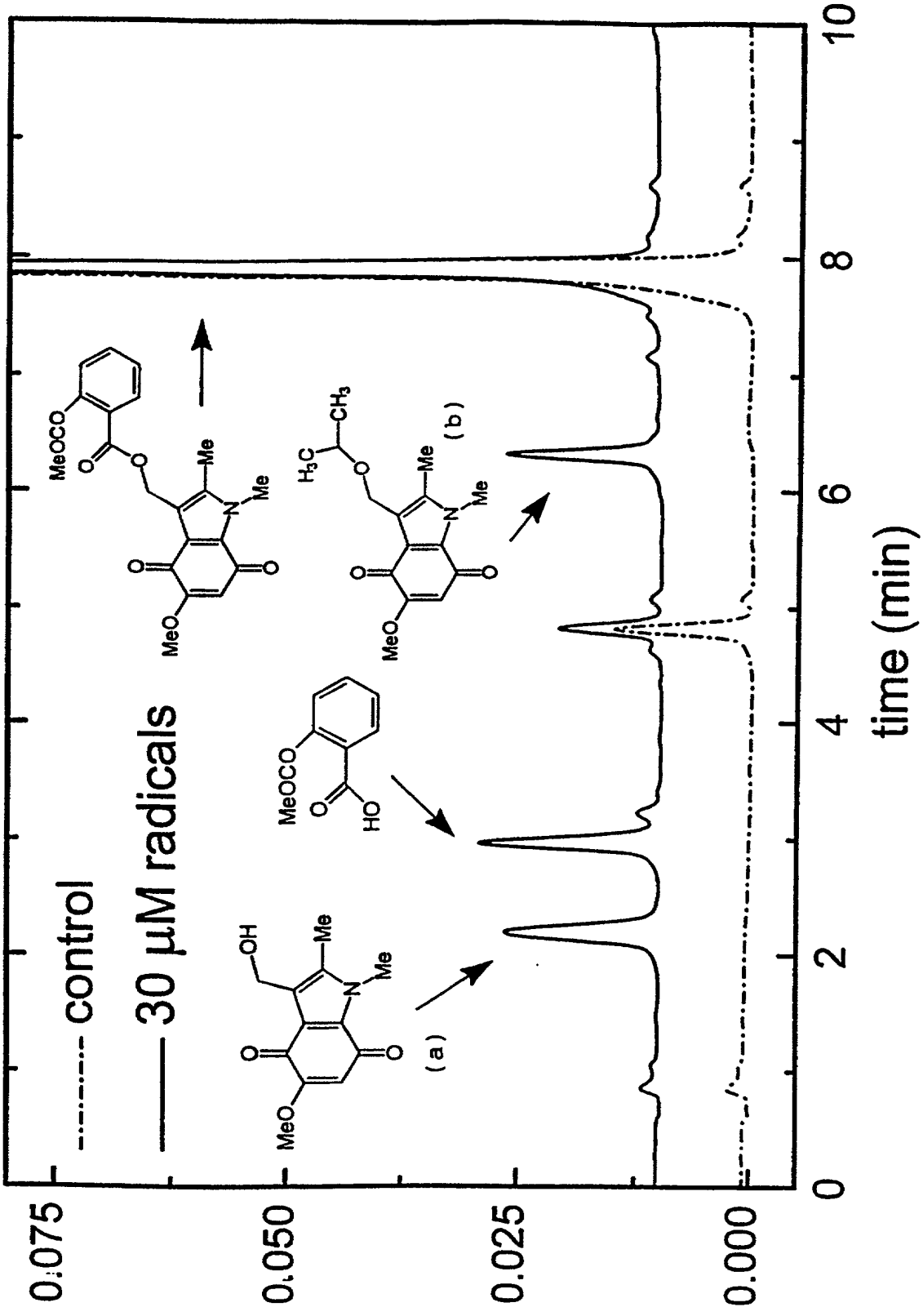

absorbance (232nm)
0.075
0.050
0.025
0.000
0
2
4
6
8
10
time (min)
control
30 µM radicals
MeOCO
HO
(a)
(b)
MeO
Me
OH
H_3C
CH_3

FIGURE 1

39-187
PBA/AJS/JK/D088187PUS

Nixon & Vanderhye P C (12/95)

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

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the specification of which (check applicable box(s)):

☐ is attached hereto
☐ was filed on _____ as U.S. Application Serial No. (To Be Assigned) (Atty Dkt. No. 39-187)
☒ was filed as PCT International application No. PCT/GB98/00461 on 13 February 1998
and (if applicable to U.S. or PCT application) was amended on 15 April 1999

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s):

Application Number	Country	Day/Month/Year Filed
9703002.7	Great Britain	13 February 1997
9712090.1	Great Britain	10 June 1997

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	Date/Month/Year Filed

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Prior U.S./PCT Application(s): Application Serial No.	Day/Month/Year Filed	Status: patented pending, abandoned
PCT/GB98/00461	13 February 1998	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And I hereby appoint **NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed)**, and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr. 29366; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; William J. Griffin, 31260; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329.*

1. Inventor's Signature: _____ Date: _____
Inventor: David (first) MI BLAKE (last) British (citizenship)
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(Zip Code) HP18 9RT

2. Inventor's Signature: _____ Date: _____
Inventor: Declan (first) MI NAUGHTON (last) Irish (citizenship)
Residence: (city) Badminton, South Gloucestershire (state/country) United Kingdom
Post Office Address: Petite France Cottage, Petite France, Badminton, South Gloucestershire, United Kingdom
(Zip Code) GL19 1AF

FOR ADDITIONAL INVENTORS, check box ☒ and attach sheet with same information and signature and date for each.

39-187
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Nixon & Vanderhye P.C. (12/95)

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and (if applicable to U.S. or PCT application) was amended on **15 April 1999**

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1. Inventor's Signature: _____ Date: 30/Aug/98

Inventor: **David** (first) MI **BLAKE** (last) **British** (citizenship)

Residence: (city) **Aylesbury, Buckinghamshire** GBX (state/country) **United Kingdom**

Post Office Address: **5 Chruch Street, Brill, Aylesbury, Buckinghamshire, United Kingdom**

(Zip Code) **HP18 9RT**

2. Inventor's Signature: _____ Date: _____

Inventor: **Declan** (first) MI **NAUGHTON** (last) **Irish** (citizenship)

Residence: (city) **Badminton, South Gloucestershire** (state/country) **United Kingdom**

Post Office Address: **Petite France Cottage, Petite France, Badminton, South Gloucestershire, United Kingdom**

(Zip Code) **GL19 1AF**

FOR ADDITIONAL INVENTORS, check box ☒ and attach sheet with same information and signature and date for each.

39-187
PBA/AJS/JK/C0881879US

Nixon & Vanderhye P.C. (12/95)

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I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	Date/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56 which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S./PCT Application(s): Application Serial No.	Day/Month/Year Filed	Status: patented pending, abandoned
PCT/GB98/00461	13 February 1998	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And I hereby appoint NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed), and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr. 29366; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; William J. Griffin, 31260; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329.

1. Inventor's Signature: David R Blake Date: 20 July 2001
Inventor: David (first) | MI | BLAKE (last) | British (citizenship)
Residence: (city) Aylesbury, Buckinghamshire (state/country) United Kingdom
Post Office Address: 5 Church Street, Brill, Aylesbury, Buckinghamshire, United Kingdom
(Zip Code) HP18 9RT

2. Inventor's Signature: Declan R Naughton Date: 20 July 2001
Inventor: Declan (first) | MI | NAUGHTON (last) | Irish (citizenship)
Residence: (city) Badminton, South Gloucestershire GBX (state/country) United Kingdom
Post Office Address: Petite France Cottage, Petite France, Badminton, South Gloucestershire, United Kingdom
(Zip Code) GL9 1AF

FOR ADDITIONAL INVENTORS, check box ☒ and attach sheet with same information and signature and date for each.

3. Inventor's Signature: _____ Date: _____

Inventor: Ged (first) MI ADAMS (Deceased) (last) British (citizenship)

Residence: (city) Wheatley, Oxford (state/country) United Kingdom

Post Office Address: 59 Ladder Hill, Wheatley, Oxford, United Kingdom

(Zip Code) OX33 1HY

Signature of Legal Representative of Ged Adams (his wife): _____ Date _____

Margaret (first) MI Adams (last) _____ (citizenship)

Residence: (city) _____ (state/country) _____

Post Office Address: _____

(Zip Code) _____

4. Inventor's Signature: _____ Date: _____

Inventor: Ian (first) MI STRATFORD (last) British (citizenship)

Residence: (city) Hope Valley, Derbyshire (state/country) United Kingdom

Post Office Address: Bretton Cottage, Bretton, Eyam, Hope Valley, Derbyshire, United Kingdom

(Zip Code) S32 5QD

5. Inventor's Signature: Christopher Morris Date: 23rd July 2001

Inventor: Christopher (first) MI MORRIS (last) British (citizenship)

Residence: (city) Chippenham, Wiltshire (state/country) United Kingdom

Post Office Address: 36 Boundary Road, Chippenham, Wiltshire, United Kingdom

(Zip Code) SW15 3NN

6. Inventor's Signature: _____ Date: _____

Inventor: Mohammed (first) MI JAFFAR (last) British (citizenship)

Residence: (city) Withington, Manchester (state/country) United Kingdom

Post Office Address: 12 Everett Court, Everett Road, Withington, Manchester, United Kingdom

(Zip Code) M20 3DT

7. Inventor's Signature: _____ Date: _____

Inventor: Matthew (first) MI NAYLOR (last) British (citizenship)

Residence: (city) West Cheshunt, Hertfordshire (state/country) United Kingdom

Post Office Address: 16 The Firs, Hammondstreet Road, West Cheshunt, Hertfordshire, United Kingdom

(Zip Code) EN7 6UD

3. Inventor's Signature: _____ Date: _____

Inventor: Ged (first) MI ADAMS (Deceased) (last) British (citizenship)

Residence: (city) Wheatley, Oxford GBX (state/country) United Kingdom

Post Office Address: 59 Ladder Hill, Wheatley, Oxford, United Kingdom

(Zip Code) OX33 1HY

Signature of Legal Representative of Ged Adams (his wife):

_____ Date _____

Margaret (first) MI Adams (last) (citizenship)

Residence: (city) _____ (state/country) _____

Post Office Address: _____

(Zip Code) _____

4. Inventor's Signature: Ian Stratford Date: 29.11.99

Inventor: Ian (first) MI STRATFORD (last) British (citizenship)

Residence: (city) Hope Valley, Darbyshire GBX (state/country) United Kingdom

Post Office Address: Bretton Cottage, Bretton, Eyam, Hope Valley, Darbyshire, United Kingdom

(Zip Code) S32 5QD

5. Inventor's Signature: _____ Date: _____

Inventor: Christopher (first) MI MORRIS (last) British (citizenship)

Residence: (city) Chippenham, Wiltshire (state/country) United Kingdom

Post Office Address: 36 Boundary Road, Chippenham, Wiltshire, United Kingdom

(Zip Code) SW15 3NN

6. Inventor's Signature: M Jaffar Jr Date: 2/12/99

Inventor: Mohammed (first) MI JAFFAR (last) British (citizenship)

Residence: (city) Withington, Manchester GBX (state/country) United Kingdom

Post Office Address: 12 Everett Court, Everett Road, Withington, Manchester, United Kingdom

(Zip Code) M20 3DT

7. Inventor's Signature: _____ Date: _____

Inventor: Matthew (first) MI NAYLOR (last) British (citizenship)

Residence: (city) West Cheshunt, Hertrordshire (state/country) United Kingdom

Post Office Address: 16 The Firs, Hammondstreet Road, West Cheshunt, Hertrordshire, United Kingdom

(Zip Code) EN7 6UD

3. Inventor's Signature: _____ Date: _____

Inventor: Ged (first) MI ADAMS (Deceased) (last) British (citizenship)

Residence: (city) Wheatley, Oxford (state/country) United Kingdom

Post Office Address: 59 Ladder Hill, Wheatley, Oxford, United Kingdom

(Zip Code) OX33 1HY

Signature of Legal Representative of Ged Adams (his wife): _____ Date _____

Margaret (first) MI Adams (last) (citizenship)

Residence: (city) _____ (state/country) _____

Post Office Address: _____

(Zip Code) _____

4. Inventor's Signature: _____ Date: _____

Inventor: Ian (first) MI STRATFORD (last) British (citizenship)

Residence: (city) Hope Valley, Derbyshire (state/country) United Kingdom

Post Office Address: Bretton Cottage, Bretton, Eyam, Hope Valley, Derbyshire, United Kingdom

(Zip Code) S32 5QD

5. Inventor's Signature: _____ Date: _____

Inventor: Christopher (first) MI MORRIS (last) British (citizenship)

Residence: (city) Chippenham, Wiltshire (state/country) United Kingdom

Post Office Address: 36 Boundary Road, Chippenham, Wiltshire, United Kingdom

(Zip Code) SN15 3NN

6. Inventor's Signature: _____ Date: _____

Inventor: Mohammed (first) MI JAFFAR (last) British (citizenship)

Residence: (city) Withington, Manchester (state/country) United Kingdom

Post Office Address: 12 Everett Court, Everett Road, Withington, Manchester, United Kingdom

(Zip Code) M20 3DT

7. Inventor's Signature: Matthew Naylor Date: 13/7/01 (17th July 2001)

Inventor: Matthew (first) MI GBX NAYLOR (last) British (citizenship)

Residence: (city) West Cheshunt, Hertfordshire (state/country) United Kingdom

Post Office Address: 16 The Firs, Hammondstreet Road, West Cheshunt, Hertfordshire, United Kingdom

(Zip Code) EN7 5UD

Nixon & Vanderhye P.C. (12/95)

39-187
PCA/AJS/JK/008618/PUS

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DRUG TARGETING

the specification of which (check applicable box(s)):

☐ is attached hereto
☐ was filed on _____ as U.S. Application Serial No. 09/367,261 (Atty Dkt. No. 39-187)
☒ was filed as PCT International application No. PCT/GB98/00461 on 13 February 1998

and (if applicable to U.S. or PCT application) was amended on 15 April 1999

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Prior Foreign Application(s):

Application Number	Country	Day/Month/Year Filed
9703092.7	Great Britain	13 February 1997
9712060.1	Great Britain	10 June 1997

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	Date/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT International applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56 which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S./PCT Application(s): Application Serial No.	Day/Month/Year Filed	Status: patented, pending, abandoned
PCT/GB98/00461	13 February 1998	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And I hereby appoint NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed), and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 32776; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Dunnie M. Byers, 33163; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr. 29366; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; William J. Griffin, 31250; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329."

1. Inventor's Signature: _____ Date: _____
Inventor: David (first) MI BLAKE (last) British (citizenship)
Residence: (city) Aylesbury, Buckinghamshire (state/country) United Kingdom
Post Office Address: 5 Church Street, Brill, Aylesbury, Buckinghamshire, United Kingdom
(Zip Code) HP18 9RT

2. Inventor's Signature: Declan McNaughton Date: 10-08-01
Inventor: Declan (first) MI NAUGHTON (last) Irish (citizenship)
Residence: (city) Badminton, South Gloucestershire (state/country) United Kingdom
Post Office Address: Petite France Cottage, Petite France, Badminton, South Gloucestershire, United Kingdom
(Zip Code) GL9 1AF

FOR ADDITIONAL INVENTORS, check box ☒ and attach sheet with same information and signature and date for each.

3. Inventor's Signature: _____ Date: _____
Inventor: Gea (first) MI ADAMS (Deceased) (last) British (citizenship)
Residence: (city) Wheatley, Oxford (state/country) United Kingdom
Post Office Address: 31 Ladder Hill, Wheatley, Oxford, United Kingdom
(Zip Code) OX33 1HY

Signature of Legal Representative of Gea Adams (his wife): _____ Date _____
Margaret (first) MI Adams (last) (citizenship)
Residence: (city) (state/country)
Post Office Address:
(Zip Code)

4. Inventor's Signature: _____ Date: _____
Inventor: Ian (first) MI STRATFORD (last) British (citizenship)
Residence: (city) Hope Valley, Derbyshire (state/country) United Kingdom
Post Office Address: Bretton Cottage, Bretton, Eyam, Hope Valley, Derbyshire, United Kingdom
(Zip Code) S32 5QD

5. Inventor's Signature: _____ Date: _____
Inventor: Christopher (first) MI MORRIS (last) British (citizenship)
Residence: (city) Chippenham, Wiltshire (state/country) United Kingdom
Post Office Address: 38 Boundary Road, Chippenham, Wiltshire, United Kingdom
(Zip Code) SW15 3NN

6. Inventor's Signature: _____ Date: _____
Inventor: Mohammad (first) MI JAFFAR (last) British (citizenship)
Residence: (city) Withington, Manchester (state/country) United Kingdom
Post Office Address: 12 Everett Court, Everett Road, Withington, Manchester, United Kingdom
(Zip Code) M20 3DT

7. Inventor's Signature: _____ Date: _____
Inventor: Matthew (first) MI NAYLOR (last) British (citizenship)
Residence: (city) West Cheshunt, Hertfordshire (state/country) United Kingdom
Post Office Address: 16 The Firs, Hammondstreet Road, West Cheshunt, Hertfordshire, United Kingdom
(Zip Code) EN7 5JD

39-167
PBA/AJS/JK/D088167PUS

Nixon & Vanderhye P.C. (12/95)

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DRUG TARGETING

the specification of which (check applicable box(s)):

- ☐ is attached hereto
- ☐ was filed on _____ as U.S. Application Serial No. 09/367,261 (Atty Dkt. No. 39-167)
- ☒ was filed as PCT international application No. PCT/GB98/00461 on 13 February 1998

and (if applicable to U.S. or PCT application) was amended on 15 April 1999

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s): Application Number	Country	Day/Month/Year Filed
9703002.7	Great Britain	13 February 1997
9712090.1	Great Britain	10 June 1997

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	Date/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56 which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S./PCT Application(s): Application Serial No.	Day/Month/Year Filed	Status: patented pending, abandoned
PCT/GB98/00461	13 February 1998	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And I hereby appoint NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed), and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Michard, 29009; Duane M. Byers, 33363; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr. 29365; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; William J. Griffin, 31260; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329."

1. Inventor's Signature: _____ Date: _____
   Inventor: David (first) MI BLAKE (last) British (citizenship)
   Residence: (city) Aylesbury, Buckinghamshire (state/country) United Kingdom
   Post Office Address: 5 Church Street, Brill, Aylesbury, Buckinghamshire, United Kingdom
   (Zip Code) HP18 9RT

2. Inventor's Signature: _____ Date: _____
   Inventor: Declan (first) MI NAUGHTON (last) Irish (citizenship)
   Residence: (city) Badminton, South Gloucestershire (state/country) United Kingdom
   Post Office Address: Petite France Cottage, Petite France, Badminton, South Gloucestershire, United Kingdom
   (Zip Code) GL9 1AF

FOR ADDITIONAL INVENTORS, check box ☒ and attach sheet with same information and signature and date for each.

3. Inventor's Signature: _____ Date: _____

Inventor:	Ged (first)	MI	ADAMS (Deceased) (last)	British (citizenship)
Residence: (city)	Wheatley, Oxford		(state/country) United Kingdom	
Post Office Address:	53 Ladder Hill, Wheatley, Oxford, United Kingdom			
(Zip Code)	OX33 1HY			

Signature of Legal Representative of Ged Adams (his wife): _____ Date _____

	Margaret (first)	MI	Adams (last)	(citizenship)
Residence: (city)			(state/country)	
Post Office Address:				
(Zip Code)				

4. Inventor's Signature: _____ Date: _____

Inventor:	Ian (first)	MI	STRATFORD (last)	British (citizenship)
Residence: (city)	Hope Valley, Derbyshire		(state/country) United Kingdom	
Post Office Address:	Bretton Cottage, Bretton, Eyam, Hope Valley, Derbyshire, United Kingdom			
(Zip Code)	S32 5QD			

5. Inventor's Signature: _____ Date: _____

Inventor:	Christopher (first)	MI	MORRIS (last)	British (citizenship)
Residence: (city)	Chippenham, Wiltshire		(state/country) United Kingdom	
Post Office Address:	36 Boundary Road, Chippenham, Wiltshire, United Kingdom			
(Zip Code)	SW15 3NN			

6. Inventor's Signature: _____ Date: _____

Inventor:	Mohammed (first)	MI	JAFFAR (last)	British (citizenship)
Residence: (city)	Withington, Manchester		(state/country) United Kingdom	
Post Office Address:	12 Everett Court, Everett Road, Withington, Manchester, United Kingdom			
(Zip Code)	M20 3DT			

7. Inventor's Signature: _____ Date: _____

Inventor:	Matthew (first)	MI	NAYLOR (last)	British (citizenship)
Residence: (city)	West Cheshunt, Hertfordshire		(state/country) United Kingdom	
Post Office Address:	18 The Firs, Hammondstreet Road, West Cheshunt, Hertfordshire, United Kingdom			
(Zip Code)	EN7 6UD			

MA 7-25-01
aka GERALD EDWARD ADAMS

3. Inventor's Signature: _____ Date: _____

Inventor:	Ged (first)	MI	ADAMS (Deceased) (last)	British (citizenship)
Residence: (city)	Wheatley, Oxford		(state/country) United Kingdom	
Post Office Address:	59 Ladder Hill, Wheatley, Oxford, United Kingdom			
(Zip Code)	OX33 1HY			

Signature of Legal Representative of Ged Adams (his wife): MA 7-25-01 Margaret Adams Date 25th July 2001

	Margaret (first)	MI	Adams (last)	BRITISH (citizenship)
Residence:	HOLTON, OXFORD		(state/country) UNITED KINGDOM	
Post Office Address:	FALLOWFIELD, HOLTON, OXFORD, UNITED KINGDOM			
(Zip Code)	OX33 1QS			

4. Inventor's Signature: _____ Date: _____

Inventor:	Ian (first)	MI	STRATFORD (last)	British (citizenship)
Residence: (city)	Hope Valley, Derbyshire		(state/country) United Kingdom	
Post Office Address:	Bretton Cottage, Bretton, Eyam, Hope Valley, Derbyshire, United Kingdom			
(Zip Code)	S32 5QD			

5. Inventor's Signature: _____ Date: _____

Inventor:	Christopher (first)	MI	MORRIS (last)	British (citizenship)
Residence: (city)	Chippenham, Wiltshire		(state/country) United Kingdom	
Post Office Address:	36 Boundary Road, Chippenham, Wiltshire, United Kingdom			
(Zip Code)	SW15 3NN			

6. Inventor's Signature: _____ Date: _____

Inventor:	Mohammed (first)	MI	JAFFAR (last)	British (citizenship)
Residence: (city)	Withington, Manchester		(state/country) United Kingdom	
Post Office Address:	12 Everett Court, Everett Road, Withington, Manchester, United Kingdom			
(Zip Code)	M20 3DT			

7. Inventor's Signature: _____ Date: _____

Inventor:	Matthew (first)	MI	NAYLOR (last)	British (citizenship)
Residence: (city)	West Cheshunt, Hertfordshire		(state/country) United Kingdom	
Post Office Address:	16 The Firs, Hammondstreet Road, West Cheshunt, Hertfordshire, United Kingdom			
(Zip Code)	EN7 6UD			

3. Inventor's Signature: _____ Date: _____

Inventor: **Ged** (first) MI **ADAMS (Deceased)** (last) **British** (citizenship)

Residence: (city) **Wheatley, Oxford** (state/country) **United Kingdom**

Post Office Address: **59 Ladder Hill, Wheatley, Oxford, United Kingdom**

(Zip Code) **OX33 1HY**

Signature of Legal Representative of Ged Adams (his wife):

_____ Date _____

Margaret (first) MI **Adams** (last) (citizenship) _____

Residence: (city) _____ (state/country) _____

Post Office Address: _____

(Zip Code) _____

4. Inventor's Signature: _____ Date: _____

Inventor: **Ian** (first) MI **STRATFORD** (last) **British** (citizenship)

Residence: (city) **Hope Valley, Darbyshire** (state/country) **United Kingdom**

Post Office Address: **Bretton Cottage, Bretton, Eyam, Hope Valley, Darbyshire, United Kingdom**

(Zip Code) **S32 5QD**

5. Inventor's Signature: _____ Date: _____

Inventor: **Christopher** (first) MI **MORRIS** (last) **British** (citizenship)

Residence: (city) **Chippenham, Wiltshire** (state/country) **United Kingdom**

Post Office Address: **36 Boundary Road, Chippenham, Wiltshire, United Kingdom**

(Zip Code) **SW15 3NN**

6. Inventor's Signature: _____ Date: _____

Inventor: **Mohammed** (first) MI **JAFFAR** (last) **British** (citizenship)

Residence: (city) **Withington, Manchester** (state/country) **United Kingdom**

Post Office Address: **12 Everett Court, Everett Road, Withington, Manchester, United Kingdom**

(Zip Code) **M20 3DT**

7. Inventor's Signature: _____ Date: _____

Inventor: **Matthew** (first) MI **NAYLOR** (last) **British** (citizenship)

Residence: (city) **West Cheshunt, Hertrordshire** (state/country) **United Kingdom**

Post Office Address: **16 The Firs, Hammondstreet Road, West Cheshunt, Hertrordshire, United Kingdom**

(Zip Code) **EN7 6UD**